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THE BIO-ASSAY OF VERATRUM VIRIDE

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Veratrum viride is one of the drugs with great activity that has not been standardized biologically, although the total alkaloidal content is found on the labels of the products of certain manufacturers; however, the drug contains several alkaloids possessing different physiological actions, so that the measure of its physiological activity could not accurately be deduced from the total alkaloidal content, chemically determined (1). As the drug has obtained wide use in the treatment of certain obstetrical conditions (eclampsia) and has been found efficacious in lowering high blood pressure, the assay by biological methods was undertaken.

METHODS OF BIO-ASSAY

The most striking clinical manifestation of veratrum viride is on the circulation, consisting of cardiac slowing and subsequent fall in the blood pressure (2). It was thought that there might be a definite ratio between the dose per kilogram of the animal and either the decrease in the heart rate or the fall in blood pressure, or both. However, the examination of a number of blood pressure tracings in dogs and cats gave little hope that the activity of the drug could be measured in this way as the results were not sufficiently uniform with a given dose, and further, on repeated dosage the cardiac slowing and fall in blood pressure is replaced by a quickening of the heart rate and a rise in blood pressure. For these reasons the method is not suitable.

As toxic doses of veratrum viride stop the frog's heart in systole, an attempt was made to determine the minimum dose that would

stop the heart in systole after the manner of digitalis. The method is suitable for the alkaloid veratrin (cevadin) which has an action similar to veratrum viride on the circulation, and for very active preparations of the drug, but with the weaker preparations of the drug the dosage is so large that the absorption is imperfect and the results, therefore, were too variable to warrant the use of this method.

The fatal dose for cats, after Hatcher's method of assaying digitalis, also proved unsuitable as will be discussed later.

The minimum fatal dose for frogs (m. f. d.) and guinea pigs was then determined, and was found to be constant for each drug within a variation of 20 per cent. The method, of course, determines only the strength of one preparation of the drug as compared with other preparations and does not give evidence for the dosage necessary to obtain results clinically. The results of the assay by determining the fatal dose for frogs will be given first, and then the results on guinea pigs.

Methods of determining the minimum fatal dose for frogs. The frogs were kept in a tank of running water (which was an inch deep at the shallowest part) at least twelve hours before they were used; the temperature of the water being about 15°C. On the morning of the injection they were placed in glass jars, with sufficient water in the jars to keep them moist, at room temperature not above 20°C. The solutions were injected into the ventral lymph sac; usually about 0.5 cc. solution for a 30 gram frog, but somewhat larger doses of the weaker preparations were required. The alcohol was evaporated from the tincture and the fluid extracts, which were then made up to the original volume with normal saline solution slightly acidulated with sulphuric acid to insure the solution of the active principles. The weaker preparations were made to a 20 per cent strength to avoid the injection of too large an amount of solution; the most active was diluted to a 5 per cent solution. The frogs were examined from time to time during the day. After death the heart was laid bare for inspection. The frog was considered to be dead when the heart stopped, usually with the ventricles in the systolic position but occasionally in the median position. After exposing the heart in a number of frogs that apparently were dead and finding the heart still beating fairly well, a routine procedure of careful examination of the thorax was established, for cardiac pulsations are transmitted to the thoracic wall and are readily seen.

The solutions examined. Eight tinctures, two fluid extracts and one Norwood tincture were examined; the latter was considered a 50 per cent tincture; four of the tinctures were bought on the market and were made by well known firms, two were made by Professor Kraemer of Philadelphia about three years previously and were obtained from the pharmacological department of Western Reserve University, as was one other tincture; one tincture was found in this laboratory and

was of unknown origin, and was not identified beyond the fact that it gave the characteristic tingling, pricking sensation when rubbed into the abraded skin. As some of the samples were three years old, the question of deterioration was considered, but evidently the tinctures keep fairly well for of two specimens obtained direct from the same manufacturer, one received over two years ago was about twice as active for frogs as the one received at the time of use.[3] The fluid extracts were different samples of the same manufacture. Two of the tinctures were made from the same specimen of veratrum viride by different individuals and gave markedly different results, which will be discussed in the next section.

The minimum fatal dose for frogs. The minimum fatal dose for frogs was considered to be the dose which killed the frog in from ten to fifteen hours, with the heart stopped in systole. Larger doses killed the frog with the heart stopped in systole, in less time, but it was thought that considering the variable factor of absorption when larger doses were required this method gave the more uniform results. The earliest effect obtained was the cessation of the respiratory movements; usually within a few minutes of the injection the respiration gradually ceased and was not resumed in the fatal doses. This effect was observed while the frog sat in the usual position, and preceded the other effects of veratrum by a few minutes. Gradually the characteristic action of veratrum on the muscles appeared so that the frog was unable to turn on its belly when placed on its back, and drew its legs up slowly after jumping; usually, after some three or four hours the paralysis was practically complete and the reflexes were lost. The heart continued to beat for several hours after paralysis was complete, except from doses well above the minimum fatal dose, when there was an early arrest. In non-fatal cases recovery may begin in five or six hours, but may be delayed much longer. Frequently after from ten to fifteen hours paralysis was complete but the heart was seen to be beating, so that these experiments were classed as non-fatal; occasionally a frog lived longer than twenty hours in such a state but did not recover. The minimum fatal dose of seven tinctures, two fluids extracts and one Norwood tincture was thus determined. The minimum fatal dose of the tinctures as given in table 1 varied from 1.2 mgm. to 6.5 mgm. of the drug per gram of frog, with a mean of 2.3 mgm. It will be noted that four of the seven tinctures lie within a range of 20 per cent of this mean; the most active being about double the mean strength and the weakest specimen having only two-fifths of the mean

strength; the other specimen (4.0 mgm.) was made from the green drug, which may account for the lower toxicity because of the presence of moisture in the drug. The possibility of variation in the strength of the tinctures is well illustrated by the following results: The fatal doses of two tinctures made from the same sample of veratrum by different individuals were determined on frogs and guinea pigs, and the strength of one was about double the other. The fatal doses for the guinea pigs were about 0.40 mgm., or somewhat above, and 0.24 mgm. per gram, respectively. The fatal doses for the frogs were 6.5 and 5.5 mgm. per gram, respectively, but as the latter dose was determined in March, when the toxicity of veratrum is but one-half that of the fall months, for comparison the quantities would be 6.5 or 3.0 mgm., or somewhat less. The tinctures were made about three years before being examined and were kept under similar conditions. The variability in the strength may be explained by the deterioration of the weaker specimen, or possibly there may have been this difference in activity originally, although both tinctures were made by capable individuals. Whatever the cause of the wide difference in potency, it is very striking and is certainly a factor in urging the necessity of standardization.

Three tinctures, said to be assayed to contain 0.1 per cent total alkaloids, gave fatal doses of 1.3, 2.3 and 2.5 mgm.; the first two were made by the same firm and the more active had been in the laboratory for two years, while the weaker one was obtained at the time of use; the variations in the fatal doses for the guinea pigs were slight.

The minimum fatal dose of the fluid extracts. The fatal dose of two fluid extracts was 2.8 and 5.5 mgm., which is considerably greater, in the latter instance, at least, than the mean of the tinctures (2.3). The Norwood tincture, considered as a 50 per cent solution, gave a fatal dose of 3.5 mgm., which is also above the mean of the tinctures. As fluid extracts of digitalis are unreliable (3), it is rather to be expected that concentrated solutions of veratrum would be proportionally weaker than the less concentrated tinctures.

Accuracy of the method. Inspection of table 1 shows that the minimum fatal dose for each preparation usually falls within fairly narrow limits; the occasional death from a dose much below the mean minimum can be disregarded. As the fatal dose is approached deaths become more frequent (as in no. 7 and no. 5) but usually the limits are within 25 per cent and more frequently 5 or 10 per cent; for practical purposes, just as with digitalis, this range of variation is practi-

TABLE 1

Fatal dose of veratrum viride for frogs, milligram of drug per gram of frog; + = fatal

TR. 1	TR. 9	TR. 2	TR. 10	TR. 10*	TR. 11	TR. 3	TR. 4	TR. 4b*	FLUID EXTRACT			NOR-WOOD	TR. V. ALBUM
									6	6*	7	5	8
0.5	1.0	1.0	1.0	1.2	1.2	2.0	1.2	2.0	1.0	2.0	1.0	1.0	0.50
0.8	1.2	1.4	1.4	2.4	1.4	3.0	1.3	3.0	1.5	3.0	1.5	1.3	0.55
1.0	1.4	1.8	2.0	3.0	1.6	3.5	1.4+	4.0	2.0	4.0	2.0	1.3	0.60+
1.0	1.5+	2.0+	2.0	3.0+	1.6	3.8	1.8	4.0	2.4	4.5	2.6	1.3+	0.60+
1.0+	1.5+	2.2	2.1	3.5	1.6+	3.8+	2.0	4.5	2.6	4.5	3.0	1.3+	0.60+
1.0+	1.6	2.2+	2.1	4.0	1.8	3.8+	2.2+	4.5	2.7+	4.5	3.4	1.4	0.60+
1.1	1.6+	2.2+	2.1+	4.0	1.8+	4.0+	2.5	5.0	2.8	5.0+	3.8	1.4	0.65+
1.1	1.7	2.2+	2.2	4.5	1.8+	4.0+	2.8	5.0+	2.8+	6.0+	4.0	1.4	0.70+
1.1+	1.8	3.0+	2.2	4.5	2.0	4.0+	3.0	5.0+	2.8+		4.2	1.5	
1.15	1.8		2.2	4.5	2.0		3.0	5.5	2.8+		4.2	1.5	
1.2+	1.8		2.2+	4.5+	2.0		3.0+	5.5+	3.0+		4.2+	1.5+	
1.2+	1.8		2.3+	5.0+	2.0+		3.0+	5.5+			4.5	1.9	
1.25+	1.8+		2.3+	5.0+	2.2		3.6	5.5+			4.5	2.1	
1.25+	1.8+		2.3+	5.5+	2.4+		3.6	6.0+			4.5	2.3	
	2.0+		2.3+	6.0+	2.5+		3.6				4.5+	2.4	
1.5	2.0+		2.4+		2.5+		4.5				4.5+	2.4+	
1.5+	2.0+		2.5+		2.5+		5.5				5.0	2.5	
1.5+							6.5+				5.0	2.5	
1.5+							6.5+				5.0+	2.5	
1.5+							6.5+				5.0+	2.5+	
											5.5+	2.7	
											5.5+	2.8	
											5.5+	2.8	
												2.8+	
												3.0	
												3.3	
												3.3+	
												3.5	
												3.5+	
												3.5+	
												3.5+	
M. F. D. 1.3	2.0	2.2	2.3	5.0	2.5	4.0	6.5	5.5	2.8	5.5	5.5	3.5	0.60

* Experiments made in March; all others in the fall months.

cally negligible, and if greater accuracy is desired it could undoubtedly be obtained by using a larger number of frogs. One source of error is the marked *seasonal variation*, as is illustrated by the following: The minimum fatal dose of the series was determined during the latter two weeks of November and the first two weeks of December, 1916.

In March, 1917, the minimum fatal dose of two of the tinctures was again determined, and also the minimum fatal dose of one of the samples of cevadin, and both were found to be double that previously obtained in the fall and early winter although the conditions were essentially the same. Thus the minimum fatal dose of the tinctures was 2.3 mgm. and 2.8 mgm. of drug per gram of the frog in early December, but was 5.0 mgm. and 5.5 mgm. respectively in March. The end point, as regards the position of the ventricles, was not as clearly defined as in the March frogs. The frogs were in average condition in March but were somewhat less active than in the fall months. The temperature of the water was similar and it was found, further, that there was little difference in the dose in frogs kept in water at 13°C. and 16°C. This seasonal variation could be met by comparing the fatal doses of the drug with the dose of veratrin after the manner of comparing the minimum fatal dose of digitalis with that of ouabain. Unfortunately the minimum fatal dose of veratrin was not determined in the fall months but as the dose that caused systolic arrest within an hour lay within narrow limits, presumably the fatal dose could be determined readily. The fatal dose of one specimen of veratrin as determined in March, was about 0.007 mgm. per gram, but the end point was not as sharply defined as in the fall months; judging from veratrum, the dose in the fall would be about one-half this, or approximately 0.0035 mgm. per gram.

The minimum systolic dose (*m.s.d.*). This is the minimum dose which stopped the heart in systole with the auricles dilated and either feebly beating or quiescent, after the manner of digitalis. The dose varies within narrow limits for the alkaloid veratrin, which has a very similar action to veratrum viride on the heart, and for the more active preparations of veratrum but not for the weaker preparations. The minimum systolic dose for six samples of the alkaloid lay between the extremes of 0.028 mgm. and 0.037 mgm. per gram (0.028, 0.029, 0.030, 0.032, 0.035, 0.037) with an average of 0.032 mgm.[1] (see table 2).

Fewer experiments were made in determining the minimum systolic dose of the different alkaloids than were made in determining the minimum fatal dose of the Galenic preparations, but they indicate

[1] Three preparations were of the alkaloid and three of the alkaloid sulphate, but the dose is given as the weight of each, disregarding the sulphate radical, for no essential difference was found in the results. It happened that the most active preparation was the sulphate, and the weakest the alkaloid. The alkaloids were acidulated with sulphuric acid to bring them into solution.

that the variations are less than with the latter method and the end point is more easily determined; in fact, it would be difficult to determine from inspection of the heart only whether a veratrin or a digitalis drug had been administered. One tincture caused arrest of the heart in systole (m.s.d.) with 4.3 mgm., with very little variation of dosage

TABLE 2

The quantity of the alkaloid veratrin causing systolic arrest of the frog's heart in one hour and the effect of alcohol on the size of the dose; doses in milligram per gram; + = positive result in one hour

Aklaloid	1103	A*	1233*	C	D*	B
Normal saline	0.026	0.028	0.027	0.028	0.028	0.030
	0.028+	0.029+	0.030+	0.032	0.032	0.032+
	0.030+	0.030+	0.030+	0.034	0.035	0.032+
	0.032+		0.035	0.035+	0.037+	0.032+
	0.032+			0.035+	0.039+	0.033+
						0.035+
25 per cent alcohol	0.030+	0.029+	0.025			0.025
	0.032+	0.031+	0.027+			0.030
	0.034+	0.032+	0.030+			0.032+
			0.035+			0.033+
						0.033+
						0.035+
						0.040+
50 per cent alcohol	0.020	0.023	0.020+			0.020+
	0.022	0.024+	0.022+			0.023
	0.023		0.025+			0.025
	0.025+		0.030+			0.025+
	0.030+					0.025+
						0.028+
						0.030+
						0.032+
						0.033+
						0.035+

* The pure alkaloid; the others were the sulphate of the alkaloid.

in different frogs, while the fatal dose was 2.5 mgm. One fluid extract with a large fatal dose (5.5 mgm.) did not cause systolic arrest with over 20.0 mgm., nor did the Norwood tincture with double the fatal dose, presumably because absorption was imperfect as the dose was quite large and a large amount of extractive matter was also injected.

The determination of the minimum systolic dose, therefore, is not suitable for general use because of the inaccuracy of the results with the weaker Galenic preparations, although it is quite accurate for assaying the alkaloids and the more active Galenicals. The advantages over the minimum fatal dose method, however, are not sufficiently great to apply the method to the tinctures and the fluid extracts by extracting their alkaloids to remove the "extractive" matter and to permit concentration of the quantity of fluid injected.

The effect of alcohol. As the tinctures and the fluid extracts are made with alcohol, a number of experiments were made to determine the

TABLE 3

Fatal dose of veratrum viride for guinea pigs in milligram of the drug per gram of pig; + = fatal in minutes

TR. 1	TR. 10	TR. 2	TR. 4	TR. 4b	FLUID EXTRACT		NORWOOD 5	TR. VER. ALBUM 8
					7	6		
0.03	0.07	0.10	0.16	0.20	0.12	0.16	0.10	0.025+97′
0.04	0.08	0.14	0.24	0.20	0.16	0.24	0.14	0.030+18′
0.05	0.10	0.20	0.28	0.24+70′	0.20	0.30	0.16	
0.07	0.11	0.26	0.36*	0.28+30′	0.24	0.36	0.20	
0.10	0.14	0.32		0.32+30′	0.30	0.42+60′	0.25	
0.15	0.16	0.32		0.35+20′	0.32		0.30	
0.15	0.18	0.36+50′		0.40+12′	0.36		0.32	
0.165	0.20+25′				0.36+30′		0.35+20′	
0.17+160′					0.40+50′		0.37+37′	
0.17+35′					0.44+30′			
0.18+25′					0.96+15′			
0.18+25′								
0.20+20′								

* Very close to fatal dose.

effect of this factor on the assay. The systolic end point was determined with four of the alkaloids dissolved in acidulated saline and in 25 and 50 per cent alcohol. The 25 per cent alcohol was without influence on the end results but the 50 per cent alcohol lowered the minimum systolic dose from 10 to 30 per cent. The larger percentage of alcohol also materially increased the toxicity of the tinctures so that, in the determination of the fatal doses, only the solutions from which the alcohol had been evaporated were used.

The minimum fatal dose (m.f.d.) in guinea pigs. This was taken as the smallest dose which caused death in about one-half to two hours. Larger doses resulted in death in from thirty minutes to a minimum of from ten to twelve minutes. Death after two hours was very infre-

quent. The usual time of death was from twenty-five to sixty minutes after the injection; doses somewhat larger than the minimum may not materially hasten the fatal termination, probably because of the variations in the rate of absorption. The minimum fatal dose of the tinctures lay between 0.17 and 0.40 mgm. (0.17, 0.20, 0.24, 0.36, 0.40) of the drug per gram of guinea pig; of the fluid extracts, 0.36 and 0.40 mgm., and of the Norwood tincture, 0.35 mgm. (see table 3). This method is accurate within the limits of 20 per cent or probably much less, which is sufficient for this type of assay. For practical work it would be best to have this dose kill three or four of five animals; this was done with but one tincture in this series for the supply of pigs did not permit more intensive investigation; however a glance at the table will show that the minimum dose was determined within narrow limits in the others. There were very few abnormal experiments, i.e., fatalities from doses below the mean minimum or survivals from doses much above the mean minimum; too much stress cannot be placed on this statement without a larger number of experiments, but the absence of these abnormalities is rather striking. The fatal doses of the veratrin alkaloids were not determined.

The method for guinea pigs. The solutions were the same as used in the frog work. Injections were made intraperitoneally to insure more uniform absorption; usually about a total quantity of 2.0 cc. was injected. In non-fatal experiments the pigs were used a second time, after a lapse of at least four weeks when the weight was somewhat above the former weight. No increased susceptibility to the second dose was noted.

To illustrate the symptoms of poisoning by veratrum, the following note of one experiment will be given.

12.52. Respiration 130 per minute; pig injected intraperitoneally.

12.55. Nausea and retching; respiration slowed.

12.57. Respiration 50 per minute and very irregular or jerky; continual retching and marked salivation.

1.00. Very quiet, but vomits several times a minute; respiration is 35 per minute; heart irregular and pounding.

1.06. Very quiet; lying on belly but turns over when placed on back; respiration very shallow and infrequent and just a mere jerk.

1.20. Quiet; respiration very shallow and infrequent; is not vomiting.

2.20. Convulsive movements; heart very irregular and respiration infrequent; gasping; lies on belly and paws the air; grits teeth; hair is "ruffled."

4.00. Improving; respiration is now regular but still somewhat infrequent. (The pig died during the night.)

The "bucking" that is said to be rather characteristic of veratrum was seen but once in this work. Very infrequently there was seen a slight degree of the

same action, which is best styled as "starting," for the animal seldom left the floor in the convulsive movements. With large doses erection of the hair or "ruffling" was frequent. When death occurred in an hour or two, the symptoms were similar to those described above but more severe.

Comparison of fatal doses for frogs and guinea pigs. To simplify this comparison, the fatal doses have been plotted in figure 1. In general it may be said that with but one exception the two run a fairly parallel course, i.e., the larger the fatal dose for the frog, the larger the dose for the pig, or vice versa. The one striking exception is the tincture (no. 2) of unknown origin. The reason for this variation is not at hand, and there is not sufficient of the tincture to investigate the cause of the difference in the reaction of the two species.

The minimum fatal dose for cats. An attempt was made to determine the fatal dose for cats of the veratrin alkaloid, administered slowly by vein, according to Hatcher's (4) method of standardizing the digitalis drugs. A priori the method would not seem as suitable for drugs of the veratrum class as for digitalis, for death from the latter is usually due to overstimulation of the cardiac muscle while the veratrum death is due to respiratory failure although, under artificial respiration, cardiac failure seems to be a very important, if not the chief factor in fatality. The end point is not sharply defined as it is with digitalis, i.e., there is considerable margin between the dose which causes very marked cardiac depression and death. In other work we have noticed that after the blood pressure has fallen to a very low level, quite large doses of veratrum are needed to cause death. In a series of eight experiments the minimum fatal dose for cats was far from uniform, so that in our hands, at least, the method seems unsuitable for bio-assay of veratrum. The results are given in table 4.

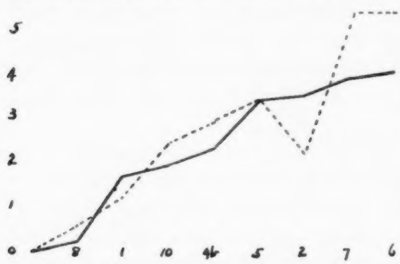

Fig. 1. Comparison of the fatal doses of veratrum for frogs and guinea pigs. ——— represents the dose for frogs, ... for guinea pigs. The ordinates are the fatal doses for frogs in milligram per gram and for guinea pigs in 0.1 mgm. per gram. The abscissa—the number of the preparations tested; 1, 10, 4b, 2 are the tinctures; 5, the Norwood tincture; 6 and 7, the fluid extracts; 8, the tincture of veratrum album.

The experimental procedure and results. The cats were etherized; a tracheal cannula inserted; the vagi divided, in later experiments, as Macht and Colson

(5) obtained more uniform results with Hatcher's method with the divided vagi; the cats were then pithed through the foramen magnum and artificial respiration maintained until death. Without artificial respiration death would have resulted from respiratory paralysis and the results would have been similar to the guinea pig method and would have no advantages over that method. The veratrin was injected slowly into the femoral vein while the heart was carefully palpated or auscultated; on the appearance of slight cardiac irregularity the injection was stopped. After a few minutes the heart became regular and the injection was repeated. Rapid injection of the solution caused marked cardiac weakening and irregularity, even with doses far below the fatal quantity. It was aimed to give the solution at such a rate that the heart would stop in about an hour or an hour and a half. The alkaloid was administered in a 1-1000 solution in normal saline.

TABLE 4

Fatal dose of veratrin for cats, administered by vein, in milligram per kilo

EXPERIMENT NO.	VAGI	VERATRIN	TIME OF DEATH, IN MINUTES
2	Intact	0.70	40
Ia	Intact	84.0	43*
		109.0	50*
7	Intact	24.7	67
9	Divided	6.7	36
6	Divided	5.0	50
I	Divided	23.0	60
8	Divided	22.0	70
5	Divided	11.0	91

* Not fatal.

Choice of method. Were veratrum used as a respiratory stimulant, either the cat or the guinea pig method would have advantages for depression and paralysis of the respiratory center is the cause of the death of these animals,and the determination of the quantity of the drugs which caused respiratory failure in mammals would have obvious advantages; for this means the guinea pig would be as suitable as the cat. Under artificial respiration the hourly fatal dose of veratrum viride, administered by vein, is variable, so that this method may be considered unsuitable. For all practical purposes the fatal dose for frogs gives satisfactory results. While it merely gives the strength of one preparation of veratrum viride as compared with another, it does this within fairly narrow limits. The arguments for and against the use of frogs for the purpose of standardization have been thoroughly discussed elsewhere (6) under the bio-assay of digitalis, so this ques-

tion need not be entered into in this paper. While there are marked seasonal variations, this difficulty can be overcome by comparing the fatal doses of veratrum viride with that of cevadin, just as digitalis is compared with ouabain as a standard. Individual susceptibility simply requires the use of a larger number of frogs.

Veratrum album. But one tincture of veratrum album was obtainable so that, while it was more toxic than any of the viride solutions, conclusions cannot be drawn relative to the activity of the two species of veratrum until further samples are on hand. The minimum fatal dose for frogs and guinea pigs was 0.6 mgm. and 0.025 mgm. per gram, respectively. (But two experiments were made on the guinea pig, with doses of 0.025 and 0.03 mgm. per gram, fatal in ninety-seven and eighteen minutes respectively.)

CONCLUSIONS

Tinctures and fluid extracts of veratrum viride vary considerably in activity, as determined by the fatal dose for frogs and guinea pigs.

As the fatal dose for frogs and guinea pigs lies within narrow limits of error, it is suggested that the determination of this dose will aid in obtaining preparations of veratrum of uniform activity. For practical purposes, the fatal dose for frogs would seem to be satisfactory.

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THE PRODUCTION IN DOGS OF A PATHOLOGICAL CONDITION WHICH CLOSELY RESEMBLES HUMAN PELLAGRA[1]

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One of the most important advances in the physiology of nutrition has been the recent appreciation of the significance of factors hitherto ignored or unrecognized. Prominent among these factors stands the class of undefined substances, called by various names, such as "vitamines," etc., the presence of which in foodstuffs apparently determines whether normal nutritional rhythm shall prevail. The absence or deficiency of these substances may lead to a variety of metabolic disturbances which have been designated by different names as beri beri, scurvy, pellagra, etc., and which may be grouped together under the term "deficiency diseases."

"To give any clear and succinct clinical description of a disease like pellagra is beset with no little difficulty. The malady is so protean in its manifestations and often so varied in its evolutions as to make a clear and logical description of it by no means easy" (1). On the other hand, certain features are distinctive and the manifestations of disease may be indicated by the following descriptive phrase, "diarrhoea, dermatitis, delirium, death." In some cases constipation may be seen instead of diarrhoea, and dermatitis or delirium may be lacking. Various lesions may occur in the alimentary tract, liver and kidneys, and in the nervous tissues.

The present communication describes the experimental production in dogs of a pathological condition which closely resembles in its sym-

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tomatology pellagra in the human subject. The disease may be induced in dogs by the ingestion of a diet containing boiled peas as the chief source of nitrogen. Under suitable conditions these nutritional disturbances may be manifested even when raw meat is included in the diet. By quantitative variations in the food intake the condition may be produced in varying periods of time ranging from one month to six or eight months.

The onset of the pathological symptoms is generally very sudden. Usually the first abnormal manifestation is a refusal to eat, and examination will reveal nothing to account for the loss of appetite. The animal lies quietly in its pen and is apathetic. After continued refusal to eat for a day or two, the mouth of the dog will present a peculiar and characteristic appearance. The inner surface of the cheeks and lips and the edges of the tongue are so covered with pustules as to give the impression of a mass of rotten flesh. The odor from these tissues is foul and almost unbearable. When stroked with absorbent cotton the mucous lining of the mouth comes away in shreds. Intense salivation is present. The teeth appear to be solid and normal. A bloody diarrhoea is present, attempts at defecation being very frequent and resulting in the passage of little more than a bloody fluid of foul odor. In some cases, the thorax and upper part of the abdomen may contain many pustules half an inch in diameter which are filled with pus organisms. No other skin lesions are prominent. Death usually results without any particularly striking features.

Certain dogs presented symptoms which were not common to all but undoubtedly bear a relationship to the usual manifestations. One animal apparently in the best of health was seized with a convulsion and died in a short time. Another developed the same type of convulsions but lingered for a period of a day, during which convulsions occurred at the rate of every five or six minutes. In the earlier part of this period the animal exhibited a blind, staggering gait, and would run her nose into a wall as though she could not see it. The left eye was closed and twitching, and the left legs were not under complete control, the toes being doubled up. The right side seemed normal. During the latter part of the day the dog lay in a state that appeared to be a mild continuous spasm. A third dog showed a single convulsion as the initial symptom, and on the following day the characteristic foul mouth and bloody diarrhoea were in evidence.

At autopsy two types of conditions are recognizable. In the animals presenting foul mouth and bloody diarrhoea the chief interest centers in the lower bowel and rectum which exhibit an intense hemorrhagic appearance. With those animals dying rapidly from convulsions the only visible abnormality of the alimentary tract is the presence in the duodenum of one or more large ulcers.

OBSERVATIONS ON DOGS FED ON DIETS CONTAINING BOILED PEAS

Series 1

Five dogs maintained upon diets consisting of boiled peas, cracker meal and salad oil (cotton seed oil) exhibited typical manifestations of the diseased condition which is considered here to resemble closely human pellagra.

Methods. Immediately upon their admittance to the laboratory the experimental animals were thoroughly washed with soap and water containing an insecticide (kerosene oil). They were bathed three times a week to prevent annoyance from fleas and possible infection from this source. During a portion of the time they were kept in metabolism cages for the purpose of urine and feces collection. Nitrogen balance and protein and fat utilization trials were carried through to gain an idea of the nutritive condition of the dogs. Following these periods the animals were allowed to run out of doors in a large enclosure covered with grass and surrounded by high walls. When not in the yard the dogs were kept in a large well lighted and well ventilated room. The floor and sides of this room were covered with galvanized iron which made it possible to flush with water. The iron floor was covered with a layer of sawdust which was renewed daily. The room was divided into a number of large pens by means of iron lattice work partitions, thus securing a maximum of light and air. Each pen was occupied by one animal only. Throughout the experiment extreme care was taken to observe strict cleanliness. Whenever meat was employed, it was bought as fresh as possible. After being freed from connective tissue and adhering fat it was ground in a meat hasher, thoroughly mixed, analyzed for nitrogen and ether extract and weighed into portions, one of which constituted the meat for a dog's meal. Each portion was tightly wrapped in oiled paper. These packages were then placed in an iron pail fitted with a tight cover. The pail was sunk in an ice-salt mixture by means of which the meat was frozen. Meat for each meal was thoroughly thawed, without heating, before

being fed. While meat will keep for a long time by this method, only enough to last short periods was ever prepared.

During the balance periods catheterization was practiced daily at a definite hour. To prevent the induction of cystitis the bladder was twice irrigated with warm sterile water after the urine had been drawn off and then washed with a warm saturated solution of boric acid, a few cubic centimeters of which were allowed to remain in the bladder.

The animals were fed regularly twice daily at 9 a.m. and 4 p.m. and were given water ad libitum. The feces were marked off in periods by means of capsules of lamp black and analyzed for ether extract and total nitrogen. The urine was subjected to a determination of the following constituents, total nitrogen, urea nitrogen, ammonia nitrogen, creatinine nitrogen and phosphates. The hair and skin sloughings were analyzed separately for total nitrogen. The calorific value of the various food constituents was calculated from Bulletin 28, U. S. Department of Agriculture. Total nitrogen was determined according to the Kjeldahl-Gunning method and ether extract was estimated by the Soxhlet procedure. Urea nitrogen, ammonia nitrogen and creatinine nitrogen were determined by the respective methods of Folin (2). Titration with uranium nitrate, using potassium ferrocyanide as indicator, was employed to estimate phosphates.

Whenever mention is made of bread in the following pages, baker's white bread is to be understood. The rice fed was of the flaked variety and was always well cooked. The ordinary dried peas fed were well soaked in water and then thoroughly cooked. They were allowed to soak in just enough water so that after boiling the mixture was a semi-solid pasty mass. The length of boiling varied from one-half to one hour. Peas were cooked for each meal only so that stale or sour food could play no rôle in the production of the diseased condition under discussion.

Bitch 6 was a young, fullgrown fox terrier weighing 5.6 kilos. When procured on July 26 she was in splendid nutritive condition. From July 26 to July 29 a diet consisting of boiled peas, cracker meal and cottonseed oil was fed and the dog was given the freedom of the yard. The following food was given on July 29 and forward: 100 grams peas, 24 grams cracker meal and 9 grams cotton seed oil. The food per day contained 0.75 gram nitrogen and 96 calories per kilo body weight. The first balance period extended from August 2 to August 12 with no change in the food intake. A positive nitrogen balance of 0.054 gram nitrogen per day was obtained. The body weight remained unchanged. (*See bitch 6, period 1, page 45.*)

At the conclusion of the first balance period the food intake was reduced per

day to 30 grams peas, 60 grams cracker meal and 21 grams cotton seed oil, containing 0.40 gram nitrogen and 96 calories per kilo body weight. With this change in diet the animal was allowed her freedom out of doors. The condition of the animal was apparently normal until August 26, when she refused to eat. Examination revealed that the dog was covered with fleas. Prompt relief was afforded by baths and the liberal use of insecticides. For the next two days the animal appeared very ill and refused all food. Vermifuge and castor oil were administered without change of condition. The animal died on August 29. On autopsy nothing abnormal could be observed except that the inner surfaces of the lips were fairly covered with pustules.

Bitch 7 was of bull extraction and weighed 9.3 kilos. She was young, had borne young and was in splendid nutritive condition when procured. From July 25 to July 29 the daily food consisted of adequate quantities of a mixture of boiled peas, cracker meal and cotton seed oil. In the interval between these dates the dog was given her freedom in the yard. From July 29 the folllowing diet was given per day: 166 grams peas, 40 grams cracker meal and 14 grams cotton seed oil, containing 0.75 gram nitrogen and 96 calories per kilo body weight. The first balance period was begun on August 2 without alteration in food intake. On August 5 the bitch refused to eat. A large number of worms (ascaris) were passed on this day. The period was interrupted at this point and a large dose of vermifuge given which was followed by the passage of a great many ascaris worms. The animal did not seem to thrive well so was replaced by another bitch (*7a*) on August 14. Inspection of the table of balance period of dog 7 for four days shows a plus balance of 0.38 gram nitrogen per day. (*See bitch 7, period 1, page 46.*)

Bitch 7a. This animal was a young but fullgrown hound, weighing 15.0 kilos and in excellent nutritive condition. From August 15 the food given per day consisted of 80 grams peas, 160 grams cracker meal and 56 grams cotton seed oil, and contained 0.40 gram nitrogen and 96 calories per kilo body weight. The dog was given the freedom of the yard. On August 26 food was refused. Examination revealed the presence of great numbers of fleas, the majority of which were destroyed by bathing and by the liberal application of insecticides. After this the dog appeared in a more normal condition for a day or two, but on August 29 food was again refused. The animal was offered a little white bread which was eaten but without evident relish. On August 30 she ate a small amount of bread. The animal appeared well. From August 31 to September 7 various kinds of vegetable food were offered such as cornmeal fried in lard, bread fried in lard, oatmeal fried in lard, etc. They were merely sniffed at and perhaps tasted but very little was eaten. On September 7 the following diet was given per day and was eaten with apparent relish: 200 grams bread, 200 grams milk, 48 grams lard, which contained 0.30 gram nitrogen and 71 calories per kilo body weight. The weight had fallen from 15 kilos to 13.9 kilos. Up to September 13 the condition of the dog appeared to be more normal although the weight had decreased to 13.5 kilos. On this date no food was eaten. Examination revealed the presence on the inner surface of the lips of numbers of large pustules. The odor from the mouth was intensely offensive and was similar to that of putrefying flesh. A mixed diet was offered but was refused. The mouth was thoroughly irrigated with antiseptic solutions. Intense salivation was present and upon

lightly stroking sheets of mucous membrane were peeled off. The reason the animal refused to eat was that to do so apparently caused pain, for when fed meat broth with a spoon it was greedily devoured. The stools, diarrhoeal and containing blood, were very offensive in odor. On September 15 vomiting occurred. The vomitus was very slimy and contained small patches of mucous membrane. The animal was killed on September 16. The intestine was much congested in spots as was the omentum, spleen and pancreas. The mouth, just within the lips, appeared to be almost in a state of putrefaction. Bacteriological examination of the mouth revealed merely the presence of pus organisms. The blood and various tissues were sterile.

Bitch 8 was a fullgrown bull dog of mixed breed in excellent condition weighing 11.2 kilos. Obtained on July 25, she received unweighed but sufficient quantities of a mixture of boiled peas, cracker meal and cotton seed oil and was allowed to run in the yard. From July 29 the food given per day consisted of 200 grams peas, 48 grams cracker meal and 18 grams cotton seed oil, containing 0.75 gram nitrogen and 96 calories per kilo body weight. The first balance period was commenced on August 2 and extended until August 12 without a change in diet. During this period the body weight remained practically constant and the dog was in almost perfect nitrogenous equilibrium. (*See bitch 8, period 1, page 47.*)

At the close of the balance period the nitrogen of the food was decreased. The new dietary contained per day 61 grams peas, 122 grams cracker dust and 43 grams cotton seed oil, giving an intake of 0.40 gram nitrogen and 97 calories per kilo body weight. The usual freedom from the metabolism cages was now enjoyed. On August 26 the animal was excessively annoyed by an influx of fleas. Freedom from this was speedily obtained by bathing and by the use of insecticides. The dog refused to eat on August 28 and was given a vermifuge which failed to demonstrate the presence of worms. The next day food was again refused. When given bread on August 29 it was eaten with a fair degree of relish. Bloody stools appeared on August 30. Various cooked foods such as cornmeal, oatmeal and bread fried in lard were offered but refused for the most part. This condition continued unchanged until September 5 when the animal was given a diet consisting of fresh meat, cracker meal and lard in such proportions that the nitrogen intake was 0.30 gram and the fuel value 80 calories per kilo body weight. The next day the food was scarcely touched. The stools were watery and contained blood. On September 7 the diet was changed to 144 grams bread, 180 grams milk and 34 grams lard, containing 0.30 gram nitrogen and 71 calories per kilo body weight. The weight had fallen to 9.9 kilos. From this day to September 14 the dog appeared to improve in some respects and the weight rose to 10.1 kilos. The diarrhoeal and bloody stools still persisted. On September 14 all food was refused and the animal appeared very ill. She lay in her cage and hardly responded when called, a most unnatural occurrence since she was the most active of all the group of dogs. The mouth of the dog had somewhat the appearance described for the two previous animals, although it was not in such bad condition. Thorough cleansing was carried out each day with various antiseptics, such as warm boracic acid solution. The condition of the mouth prevented her from eating so meat broth was fed from a spoon. Vomiting occurred on September 15 and the vomitus was slimy, thick and appeared to contain small pieces of epithelium. The stools were watery, bloody and intensely

offensive in odor. The dog was killed on September 16. The small and large intestines were much inflamed. The appearances in this dog were much less prominent than in the previous animals of this group. The bacteriological examination showed that the tissues were sterile. The mouth contained the ordinary pus organisms.

Bitch 9 was a short-haired mongrel, fullgrown and weighing 12 kilos. She was in very good nutritive condition. When first obtained (July 25) she was fed a mixture of boiled peas, cracker meal and cotton seed oil without regard to the exact quantities, and allowed the freedom of the yard. The diet was accurately weighed from July 29 forward, and the quantities of food given per day were as follows: 214 grams peas, 52 grams cracker meal and 19 grams cotton seed oil. The daily food yielded 0.75 gram nitrogen and 96 calories per kilo body weight. Upon this diet the first balance period was begun on August 2 and ended August 12. During this interval a positive balance of 0.45 gram nitrogen per day resulted. The body weight increased 0.4 kilos. (*See bitch 9, period 1, page 48.*)

At the conclusion of the balance period the diet was diminished, the dog receiving per day 66 grams peas, 132 grams cracker meal and 47 grams cotton seed oil, containing 0.40 gram nitrogen and 96 calories per kilo body weight. The regular interval of rest from the metabolism cages was allowed. Up to August 26 the progress of the experiment was uneventful. On that day a large number of fleas were found upon the dog and she was given a thorough bathing and a liberal application of insecticides, which appeared to have the desired effects. On August 29 the regular food was refused, although a small quantity of bread was eaten. Several large pustules were discovered on the chest which contained pus organisms. Diarrhoea was prominent on August 30 and for the following days. Various foods, such as fried cornmeal, fried oatmeal and fried bread were offered the dog, but only small portions were eaten. There was a slight but steady loss in body weight and a noticeable change in the attitude of the animal. She was no longer active and playful but lay quietly or moved about slowly and with drooping ears and tail. On September 5 all food was refused. The diarrhoea was much worse. Bread and milk were eaten with relish on September 6, although the quantities consumed were small. The following food was eagerly devoured on September 7: 144 grams bread, 180 grams milk and 34 grams lard, containing 0.30 gram nitrogen and 71 calories per kilo body weight. The weight had fallen from 12.4 kilos to 9.9 kilos. No change occurred until September 14 when all food was again refused. Examination showed that the mouth of the dog was in a very foul condition. The membrane on the inner surface of the lips brushed off when washed with sponges moistened with an antiseptic solution. Intense salivation was present and diarrhoeal stools containing blood were passed. Meat broth was fed by spoon and accepted with apparent relish. Vomiting occurred on September 14. The next day, September 15, the animal was found dead. Autopsy revealed appearances similar to those obtained with dogs 6, 7 and 8. The bacteriological examination revealed nothing abnormal.

Bitch 10 was a fullgrown English setter weighing 14.4 kilos. The food from July 25 to July 29 consisted of adequate but unweighed quantities of a mixture of boiled peas, cracker meal and cotton seed oil. In the above interval the dog

was allowed the freedom of the yard. From July 29 the food given daily was as follows: 260 grams peas, 62 grams cracker meal and 23 grams cotton seed oil, containing 0.76 gram nitrogen and 97 calories per kilo body weight. The first balance period extended from August 2 to August 12 without alteration in food intake. The balance for the period was plus 0.28 gram nitrogen per day. The body weight remained practically unchanged. (*See bitch 10, period 1, page 49.*)

The diet was reduced at the conclusion of the balance period, the dog receiving per day 77 grams peas, 154 grams cracker meal and 54 grams cotton seed oil, containing 0.40 gram nitrogen and 97 calories per kilo body weight. The animal refused to eat on August 26 and inspection revealed the presence of great numbers of fleas, of which the larger portion were removed by bathing and the application of an insecticide. For the next few days the dog did not appear quite normal but the food was eaten. On August 29 the regular food was refused, although white bread in small quantities was devoured. The next day even this was refused. Attempts to feed other food such as fried cornmeal, fried oatmeal and bread fried in lard met with little success, only small quantities being consumed. The presence of large pustules on the inner surfaces of the lips was discoverd on September 4. Diarrhoea was present. On this date as the animal was in an emaciated condition and apparently very ill, she was killed. Autopsy revealed two very hard puslike formations on the small intestine and two similar spots on the liver. In certain areas from the stomach to the large intestine the mucosa was much congested, as though the arterioles had been ruptured. Bacteriological examination was made of the spots on the liver and intestine. No cultures could be grown from these areas.

Summary of series 1. From the protocols outlined it may be accepted that dogs fed upon the described quantities of boiled peas, cracker meal and cotton seed oil rapidly develop symptoms of abnormal nutrition which are characteristic. Among this group of animals the onset of the pathological manifestations was sudden and almost simultaneous. The abnormal symptoms appeared soon after an invasion of fleas and at first thought one might be inclined to attribute the pathological state to infection through fleas. This argument, however, cannot be supported for in the same room close to this group of dogs was kept a second group, likewise inflicted with the same invasion of fleas but fed upon an entirely different dietary, and the latter animals gave no evidence of untoward symptoms.

It may be pointed out also that the abnormal condition of these dogs developed shortly after a decrease was made in the quantity of nitrogen ingested. A second possibility therefore suggests itself as a reason for the production of the pathological state under discussion. The reduction in the quantity of peas ingested does play a rôle in the rate of onset of the appearances, as will be shown later, but the same picture may be observed when dogs are maintained upon the higher level

of nitrogen intake which is entirely adequate for the dog as many types of experimental nutritional studies have demonstrated. *It is therefore evident that the failure of these animals to thrive upon the prescribed dietary cannot be attributed to the low intake of nitrogen per se.* This point will be referred to later.

Shortly before the appearance of the untoward symptoms the dogs were in a good nutritive condition from the standpoint of nitrogenous balance and food utilization.

Series 2

Of the possibilities which may be suggested to account for the development of the pathological symptoms under discussion one at least, namely, infection, may be excluded by a repetition of this investigation. Accordingly, after thorough cleansing and fumigation of the room, pens and cages occupied by these animals a new lot of dogs was obtained. The details of the investigation follow:

At the commencement of this second series of experiments it was suggested that the *sudden* transition from food containing animal protein to a diet exclusively of vegetable origin might play a rôle in the decline in health observed. The animals of this series were accordingly divided into two classes, one to be fed at once upon an almost exclusive vegetable diet, the diet of the other class to be *gradually* diminished in the content of animal protein and replaced by protein of vegetable origin. Dogs 18, 21, 22 belonged to the first class and dogs 16 and 17 to the second.

Lot 1. The effect of sudden transition from a diet containing meat to a strictly vegetable diet

Bitch 18 was a black and white hound in excellent condition weighing 13.6 kilos. From October 5 the animal received 105 grams peas, 166 grams cracker meal and 26 grams lard, containing 0.5 gram nitrogen and 95 calories per kilo body weight. It will be observed that with dog 18 the only food of animal origin was lard. The freedom of the yard was allowed until October 22 when the first balance period was begun without a change in the food intake. During this interval of ten days a positive balance of 0.54 gram nitrogen per day was obtained. Up to the time of this balance period the body weight had declined from 13.6 kilos to 13.1, but later rose to 13.3 kilos. At this time the dog was receiving approximately 0.51 gram nitrogen and 98 calories per kilo body weight daily. (*See bitch 18, period 1, page 50.*)

The diet was reduced on November 3 to 50 grams peas, 120 grams cracker meal and 44 grams lard per day, containing 0.30 gram nitrogen and 80 calories per kilo body weight. This dietary was maintained until November 7 when the food

given per day was as follows: 280 grams bread and 34 grams of lard. This change did not affect the nitrogen intake but reduced the fuel value from 81 calories to 80 calories per kilo. The second balance period was begun on December 6 upon the bread and lard diet. Upon the latter food the body weight had increased to 14.0 kilos, a gain of nearly 1 kilo. During this balance period a positive balance of 0.24 gram nitrogen per day was obtained. The nitrogen and fuel value of the food received per kilo per day was 0.27 gram nitrogen and 72 calories. A still further gain in body weight from 14.0 kilos to 14.5 kilos is to be noted during this period. (*See bitch 18, period 2, page 51.*)

No alteration in food intake was made until December 18. From this date the daily food consisted of 50 grams peas, 120 grams cracker meal and 44 grams lard. Since the body weight was now 14.6 kilos the dog received 0.26 gram nitrogen and 77 calories per kilo. The progress of the experiment was uneventful until December 30 when the regular diet was refused and the dog did not appear well. Examination showed the typical signs of sore mouth seen with the previously discussed dogs fed with vegetable food. She was at once placed upon a diet of meat and chopped bones which was greedily devoured. The next day all food was refused and the dog appeared in a very serious condition. Gastro-intestinal disturbances were indicated by the character of the stools, which were diarrhoeal and contained blood. This condition gradually became worse until on January 4 the animal died. No autopsy was made.

The failure of this animal to survive tends to strengthen the hypothesis that the character of the diet was the responsible factor. The resistance of this animal was evidently much greater than that exhibited by the previous dogs fed with similar food, but the final outcome was strictly typical. The death of the animal cannot be attributed to poor utilization of the food for during the first balance the nitrogen and fat utilization was 82 per cent and 95 per cent respectively, and for the second balance period the nitrogen utilization was 91 per cent, that of the fat 98 per cent, figures which compare well with the average normal dog. In the first balance period the distribution of nitrogen in the form of urea, ammonia and creatinine was normal, the daily average being 85.0 per cent, 6.0 and 3.6 per cent respectively. The figures obtained in the second balance period are somewhat higher for ammonia and creatinine. The average percentage per day of urea nitrogen in terms of total nitrogen was 80, of ammonia nitrogen 10.4 and for creatinine nitrogen 4.2 per cent. The large difference in the weight of the dried feces during the first and second balance periods is worthy of note. In the first case peas were fed, in the second the animal received bread alone for its nitrogen supply.

Bitch 21. This animal was a white bull bitch in fine nutritive condition weighing 14.0 kilos, and had been delivered of a litter of young a short time previously. From December 18 until December 30 the daily food consisted of 105 grams peas, 166 grams cracker meal and 26 grams lard, which contained 0.49 gram nitrogen and 93 calories per kilo body weight. The body weight declined slightly on this diet, so that when the food was reduced in amount on December 30 the body weight was 13.6 kilos. The new diet was composed of 58 grams peas, 150 grams cracker meal and 46 grams lard, yielding 0.35 gram nitrogen and 90 calories per kilo body weight. The first balance was begun with this diet on January 16 and extended to January 27. A positive balance of 0.03 gram nitrogen per day was

obtained, a condition of almost perfect nitrogenous equilibrium. The body weight increased from 13.6 kilos to 13.9 kilos. *(See bitch 21, period 1, page 52.)*

A still further reduction in food intake was effected at the conclusion of the first balance period. This diet contained per day 272 grams bread and 46 grams lard, having a content of 0.27 gram nitrogen and 80 calories per kilo body weight. Nothing eventful occurred until February 8 when food was refused. Examination revealed the typical signs of abnormal conditions. These consisted of the usual sore mouth and diarrhoea. In view of these findings it was deemed advisable to attempt to save the animal and to this end meat was introduced into the dietary. The latter was composed of 50 grams meat, 140 grams cracker meal and 50 grams lard, containing 0.3 gram nitrogen and 78 calories per kilo. Living upon this food for a few days resulted in the entire disappearance of all untoward symptoms. On February 13 the animal appeared normal and the second balance period was begun and extended until February 23. During this interval a negative balance of 0.45 gram nitrogen per day resulted. In spite of this negative balance the body weight increased from 14.1 kilos to 14.3 kilos. *(See bitch 21, period 2, page 53.)*

At the conclusion of this balance period the food intake was changed to 50 grams meat, 240 grams rice and 20 grams lard per day, containing 0.30 gram nitrogen and 80 calories per kilo body weight. The third balance period extended from March 13 to March 23, the food received per day during this interval consisting of 64 grams meat, 140 grams cracker meal and 50 grams lard. It contained 0.33 gram nitrogen and 77 calories per kilo body weight. On this diet there was obtained a negative balance of 0.43 gram nitrogen per day. There was a slight decrease in body weight. *(See bitch 21, period 3, page 54.)*

A changed dietary was instituted on March 25 when the food received per day was made up as follows: 66 grams meat, 240 grams rice and 20 grams lard, containing 0.33 gram nitrogen and 81 calories per body kilo weight. This diet was maintained until April 10, the beginning of the fourth balance period. On this date the morning meal was composed of 35 grams meat, 88 grams bread and 25 grams lard, and the afternoon meal consisted of 88 grams bread and 25 grams lard. The nitrogen intake per day amounted to 0.34 gram and the fuel value to 79.0 calories per kilo body weight. A negative balance of 0.13 gram nitrogen per day was obtained. There was a slight gain in body weight. *(See bitch 21, period 4, page 55.)*

After the completion of this balance period no change in the daily dietary was made until May 6 when the food noted above was replaced by 68 grams meat, 240 grams rice and 20 grams lard. The food intake per day contained 0.33 gram nitrogen and 81 calories per kilo body weight. The fifth balance period was commenced on May 8 with alteration in the food ingested. During this interval of ten days a negative balance of 0.11 gram nitrogen per day was obtained. The body weight increased from 14.3 kilos to 14.5 kilos. *(See bitch 21, period 5, page 56.)*

At the completion, May 18, of the fifth balance period the following diet was fed: For the morning meal 35 grams meat, 88 grams bread and 25 grams lard, while the meal of the afternoon consisted of 217 grams milk, 88 grams bread and 25 grams lard. The food intake contained 0.33 gram nitrogen and 77 calories per kilo body weight. On June 5 this diet was replaced by 70 grams meat, 240

grams rice and 20 grams lard, containing 0.31 gram nitrogen and 76 calories per kilo. Upon this diet the sixth balance period was run (June 5 to 15), and resulted in a negative nitrogen balance of 0.05 gram nitrogen per day, practically nitrogenous equilibrium. The body weight remained unchanged. (*See bitch 21, period 6, page 57.*)

The animal was maintained on the same diet until June 21 when she was killed by bleeding. The appearances of the body on post mortem were normal with the exception of the presence of a single small tape worm in the small intestine. The animal was very fat, the entire musculature being encased in a thick layer of adipose tissue. The fatty tissue around the kidney and in the omentum was very large in amount.

Summary—bitch 21. The table on page 66 gives a summary of the experiment with dog 21 calculated on the basis of daily averages. From this table it may be seen that in all periods except the first the nitrogen balances are negative. With the exception of periods 2 and 3, however, the minus balance is so slight that the animal was practically in a condition of nitrogenous equilibrium even though the nitrogen intake per kilo of body weight varied from 0.30 to 0.34 gram daily with the fuel value of the food varying from 76 to 89 calories per kilo. The nitrogen distribution in the urine as represented by urea nitrogen, ammonia nitrogen and creatinine nitrogen, expressed in percentages of total nitrogen, is little different from the percentages usually obtained with dogs on a higher protein diet. The percentage of ammonia nitrogen is on the average somewhat higher than normal and the urea nitrogen somewhat lower, which would naturally occur on a diminution of the total nitrogen ingested, if the laws governing the composition obtain for dog's urine as have been demonstrated for the urine of man (2). The percentage of creatinine nitrogen is practically unchanged and constant, the quantity of meat fed apparently exerting little influence upon the percentage output of this form of nitrogen. Inspection of the last two columns of the table showing the utilization of nitrogen and fat reveals but slight variations in this connection except in the first and fifth periods. The difference in the first period can be accounted for by the normally poorer utilization of a vegetable diet. The equally poor utilization in period 5 cannot be explained, for the diets in periods 5 and 6 were practically identical in kind and quantity.

The experience gained with bitch 21 corroborates that previously obtained with dogs fed on a selected vegetable diet. Only a relatively short period of time elapses with such a diet before serious symptoms of deranged metabolism are exhibited. The present experiment also

demonstrates the possibility of the animal regaining a normal nutritive condition when fresh meat is added to the diet even though the nitrogen intake and calorific value are maintained at a relatively low level. It may also be concluded that the unfavorable symptoms observed were not the result of an infection.

Bitch 22 was a black and white bull bitch in fair bodily condition weighing 12.8 kilos. From April 19 the dietary was composed of 77 grams peas, 154 grams cracker meal and 30 grams cotton seed oil, containing 0.45 gram nitrogen and 91 calories per kilo body weight. The diet remained unchanged throughout the entire course of the experiment. On May 21 the animal gave birth to four live pups, two of which were at once removed. The bitch appeared to be in fair nutritive condition at this time. The pups were allowed to remain with the bitch until June 5, when the symptoms, sore mouth, etc., were observed in the latter. This condition gradually became worse until June 28, when she was killed by bleeding. Post mortem examination revealed nothing strikingly abnormal with the exception of the foul and inflamed condition of the mouth.

Lot 2. The effect of a gradual transition from a diet containing meat to one of vegetable origin

Bitch 16. This animal was a white bull bitch in excellent nutritive condition weighing 17.8 kilos. She was young but had probably borne two or three litters of young. Since it was planned to determine the influence of a gradual transition from a dietary containing animal food to one containing none on September 29 the following rations were given per day: 68 grams meat, 438 grams milk, 290 grams bread and 28 grams lard. The daily food contained 0.5 gram nitrogen and 80 calories per kilo body weight. The meat was reduced one-half in amount on October 13 and the milk correspondingly increased without effecting any change in nitrogen intake or calorific value. The first balance period was begun on October 22 and extended to November 1. During this period a positive balance of 0.68 gram nitrogen per day was obtained. There was also a rise of 0.3 kilo in body weight. (*See bitch 16, period 1, page 58.*)

Meat was entirely eliminated from the dietary November 3. The new rations were made up of 340 grams milk, 250 grams bread and 58 grams lard, containing 0.30 gram nitrogen and 80 calories per kilo body weight. It will also be observed that in the new diet the nitrogen intake was decreased from 0.5 gram to 0.3 gram per kilo. The experiment progressed uneventfully until November 12 when the animal refused the regular food. The next day the dog had diarrhoea, vomited and the mouth presented the appearance of abnormality previously observed in other dogs. Food of all description was refused. This condition gradually became worse until death intervened on November 18. Post mortem examination revealed the usual inflamed condition of the intestines.

The single balance period with bitch 16 showed this animal to have a positive nitrogen balance of 0.68 gram nitrogen per day upon a diet furnishing 0.50 gram nitrogen and 80 calories per kilo body weight. The nitrogen utilization was 89 per cent, that of the fat 98 per cent. The percentage distribution of urea nitrogen, 85.9 per cent, ammonia nitrogen, 4.5 per cent and creatinine nitrogen,

3.0 per cent, were normal. During this period the body weight increased. In spite of the favorable outcome of this balance period, the dog did not survive sufficiently long to carry through more balance periods.

Bitch 17 was a splendid brindle bull bitch weighing 18.2 kilos. Beginning with September 29 the following daily food was given: 70 grams meat, 442 grams milk and 300 grams bread with 28 grams lard. This food contained 0.5 gram nitrogen and 80 calories per kilo body weight. As may be seen from the make-up of this dietary a gradual transition from a diet containing meat to one comprising none was planned. On this diet the dog gained steadily in weight until on October 11 the weight had reached 18.6 kilos. The meat was decreased in the dietary on October 13 to 34 grams, one-half the original quantity, and a corresponding increase in milk was made, neither change causing any alteration in nitrogen intake or the fuel value of the food. The first balance period extended from October 22 to November 1, giving a positive balance of 0.38 gram nitrogen per day. The body weight had fallen from 18.6 kilos on October 13 to 18.2 kilos on October 22. (*See bitch 17, period 1, page 59.*)

All meat was eliminated from the dietary on November 3, the nitrogen intake being also diminished. The dog received from this date 340 grams milk, 250 grams bread and 58 grams lard, containing 5.32 grams of total nitrogen and a calculated total fuel value of 1440 calories. This diet was maintained until November 19. At this time milk was eliminated so that the daily food consisted of 376 grams bread and 50 grams lard, which contained 0.30 gram nitrogen and 80 calories per kilo body weight. It will be observed that this last change in food intake involved a further diminution of the nitrogen ingested. It was observed on November 22 that the bitch, usually extremely active and full of life, was quiet and strange; her bark was peculiar and hoarse and she had but little appetite. The next day bread and milk were eaten fairly well. But little change was observed until November 25 when the typical appearances seen in the mouth of other dogs was observed. The dog's condition grew rapidly worse, bloody diarrhoea set in, and the mouth became very sore and disgustingly foul. The body weight fell from 18.2 kilos to 15.5 kilos. On December 4 she was placed upon a diet of meat and chopped bones. From this date a gradual improvement was noted although the diarrhoea persisted until December 14 and the mouth was still somewhat sore. The feces were still soft and unformed on December 18, although no blood was contained therein. The mouth was apparently normal again. Meat, chopped bones or milk were greedily devoured but bread, cracker meal, etc., were refused. The dog was practically normal on December 30 and had gained a kilo in weight, weighing at this time 16.5 kilos. On this date she was taken from a meat and bone diet and placed upon the following: 70 grams meat, 442 grams milk, 210 grams cracker meal and 10 grams lard, containing 0.5 gram nitrogen and 80 calories per kilo body weight. The food and nitrogen intake were still further decreased on January 12. The morning meal contained 221 grams milk, 90 grams cracker meal and 17 grams lard; the afternoon meal consisted of 33 grams meat, 108 grams cracker meal and 22 grams lard. The food contained per day 0.35 gram nitrogen and 80 calories per kilo body weight.

The second balance period was begun on January 30 and ended February 9 without alteration in the above dietary. During this interval a positive balance

of 0.99 gram nitrogen per day resulted. The body weight continued to increase. Through the increased body weight the nitrogen intake per kilo was 0.33 gram and the fuel value per kilo was 78 calories. *(See bitch 17, period 2, page 60.)*

The food intake was still further decreased on February 9. The new diet consisted per day of 66 grams meat, 178 grams cracker meal and 50 grams lard, containing 0.3 gram nitrogen and 71 calories per kilo body weight. This dietary was maintained until February 24 when rice replaced the cracker meal, changing the total nitrogen intake from 5.28 grams to 5.31 grams and the fuel value from 1260 to 1280 calories. The third balance period was commenced on February 27 without alteration in the food fed. A negative balance of 0.26 gram nitrogen per day was obtained. There was an increase in body weight from 17.5 kilos to 18.1 kilos. *(See bitch 17, period 3, page 61.)*

At the conclusion of the above balance period the following dietary was instituted: 64 grams meat, 178 grams cracker meal and 50 grams lard. The food contained 0.3 gram nitrogen and 70 calories per kilo body weight. This dietary was replaced on March 22 by 80 grams meat, 240 grams rice and 30 grams lard, containing approximately the same nitrogen and calories. Upon this diet the fourth balance period was begun on March 27 and ended April. 6 In spite of a noticeable gain in body weight there was obtained a negative balance of 1.47 grams nitrogen per day. During this period the dog received per day 0.29 gram nitrogen and 70 calories per kilo body weight. *(See bitch 17, period 4, page 62.)*

In view of the very large negative balance in this last balance period the nitrogen intake was slightly increased, as may be seen from the following dietary begun on April 6: The dog received for the morning meal 42 grams meat, 100 grams bread and 25 grams lard; the afternoon meal was made up of 300 grams milk, 100 grams bread and 25 grams lard. This food contained per day 0.32 gram nitrogen and 68 calories per kilo body weight. No change in food intake was made through the fifth balance period which extended from April 24 to May 4. A positive balance of 0.04 gram nitrogen per day, an almost perfect nitrogen balance, resulted. The body weight remained stationary. *(See bitch 17, period 5, page 63.)*

At the conclusion of the fifth balance period the following daily dietary was instituted: 94 grams meat, 140 grams cracker meal and 66 grams lard, containing 0.31 gram nitrogen and 70 calories per kilo body weight. The food intake was again changed on May 20. The new diet consisted of 42 grams meat, 100 grams bread and 25 grams lard for the morning meal, and the afternoon meal was composed of 300 grams milk, 100 grams bread and 25 grams lard. The food contained per day 0.32 gram nitrogen and 69 calories per kilo body weight. This food was fed during the sixth balance period which extended from May 22 to June 1. A negative nitrogen balance of 0.32 gram nitrogen per day was obtained. In spite of this the body weight increased from 18.4 kilos to 18.8 kilos. The animal received per day during this interval 0.31 gram nitrogen and 67 calories per kilo body weight. *(See bitch 17, period 6, page 64.)*

On June 1 the following food was given per day: 100 grams meat, 140 grams cracker meal and 66 grams lard, containing 0.31 gram nitrogen and 73 calories per kilo body weight. On this diet the body weight steadily increased until at the beginning of the seventh balance period the animal weighed 19.7 kilos. During this period of ten days a positive balance of 0.92 gram nitrogen per day

resulted. The body weight increased to 20.0 kilos, so that the dog received per day 0.30 gram nitrogen and 70 calories per kilo body weight. (*See bitch 17, period 7, page 65.*)

The dog was killed by bleeding on June 28. Post mortem examination showed nothing abnormal except the presence in the small intestine of perhaps twenty tape worms in a state of partial disintegration. The body was well covered with adipose tissue.

A summary of the analytical data of this experiment is given in the table on page 66. After the first period, during which the dog was in nitrogenous equilibrium, the loss in body weight was due to the onset of the typical pathological symptoms. From this time forward, however, the body weight steadily increased. In four out of seven balance periods a positive nitrogen balance was obtained. If the entire number of balance periods is averaged it will be seen that the positive balances exceed the negative balances. The utilization of nitrogen and fat correspond fairly closely with the nitrogen balance, that is, when the balance was positive the utilization was better than when the balance was negative. When the percentage distribution of the different forms of urinary nitrogen determined is considered, it will be seen that there was a tendency toward a relatively increased percentage of ammonia nitrogen and creatinine nitrogen and a decrease in the percentage output of urea nitrogen. The greater part, if not all, of this relative increase and decrease in the respective urinary constituents can be accounted for by the diminished nitrogen intake.

Summary

From the above observations it is obvious that dogs do not thrive, when fed for even relatively short periods, on the vegetable diet selected. Moreover, such a diet is positively injurious, inducing sooner or later a series of pathological changes indicative of disturbed nutritive conditions. The symptoms are peculiarly characteristic and point to abnormalities which are almost entirely confined to the alimentary canal. The pathological appearances exhibited by dogs fed on an exclusive vegetable diet can be made to disappear and the normal condition of nutritive rhythm can be reëstablished by the addition of meat to the dietary. The cause of the failure of dogs to thrive on an exclusive vegetable diet cannot be ascribed to a diminished nitrogen intake, nor to an inability to maintain nitrogen equilibrium nor to poor utilization of nitrogen and fat. Dogs rendered abnormal by vegetable food are capable of maintaining health and vigor, nitrogen

equilibrium and of increasing in body weight over a period of six months at least when placed upon a dietary containing meat. The quantity of nitrogen which may accomplish this result when meat is in the dietary varies from 0.29 to 0.33 gram nitrogen per day per kilo body weight. The fuel value per day per kilo body weight may vary from 67 to 80 calories.

It may also be concluded that infection plays no rôle in the production of the abnormal nutritive condition of the dogs. Failure of dogs to thrive upon the selected vegetable diet must therefore be ascribed to the character of the food. Moreover, it appears immaterial whether the transition from a diet containing meat to one of vegetable origin is sudden or gradual. The final outcome is the same in both cases.

THE INFLUENCE OF LARGE AND SMALL QUANTITIES OF BOILED PEAS UPON THE PRODUCTION OF SYMPTOMS OF ABNORMAL NUTRITION IN DOGS

The results reported up to this time were obtained during the years 1905–1906. In January, 1913 the study was again resumed. The first point investigated was the possibility of the production of the diseased condition in dogs fed upon peas in quantities larger than those in the previous experiments and the determination of the influence of the larger intake of peas upon the rate of the appearance of abnormal symptoms.

The experiment was begun on January 13, 1913, with four dogs which may be described as follows:

DOG	DESCRIPTION	WEIGHT
		kilos
23	Black and tan hound, male	16.2
24	Brindle and white bull, bitch	11.5
25	Black and tan hound, bitch	11.4
26	White bull, bitch	20.0

All the animals were in splendid nutritive condition and were free from skin affections. The conditions of the experiment were as nearly like those of the previous investigation as it was possible to make them. Throughout the present study no attempt was made to analyze the excreta, the physical condition of the dogs being taken as the criterion of their nutritive well being. The food given was analyzed for nitro-

gen and was fed in definite quantities twice a day, at 7.00 a.m. and at 6.00 p.m. A record was kept of the body weight, which was observed every other day. The individual daily dietaries follow:

Dog 23

	NITROGEN	CALORIES
	grams	
250 grams dried peas	10.75	910
75 grams cracker meal	1.41	298
28 grams cotton seed oil	0.00	260
	12.16	1468

or 0.75 gram N and 90 calories per kilo body weight

Dog 24

180 grams dried peas	7.74	655
50 grams cracker meal	0.94	199
20 grams cotton seed oil	0.00	186
	8.68	1040

or 0.75 gram N and 90 calories per kilo body weight

Dog 25

180 grams dried peas	7.74	655
50 grams cracker meal	0.94	199
20 grams cotton seed oil	0.00	186
	8.68	1040

or 0.75 gram N and 90 calories per kilo body weight

Dog 26

310 grams dried peas	13.33	1128
90 grams cracker meal	1.69	350
36 grams cotton seed oil	0.00	335
	15.02	1813

or 0.75 gram N and 90 calories per kilo body weight

In general the food was greedily devoured, although now and then all the animals would develop the habit for a few days of leaving small portions of the diet. Such periods were followed by others during which all the food would be eaten. From the beginning of the experiment on January 13 up to March 15, a period of about two months,

no abnormal symptoms of any sort were in evidence except that dog 23 developed a mild diarrhoea which lasted for two days (February 5 and 6) only. The body weights on March 15 were:

DOG	MARCH 15	ORIGINAL WEIGHT
	kilos	*kilos*
23	15.7	16.2
24	11.2	11.5
25	11.7	11.4
26	19.3	20.0

A comparison of these weights with the initial weights shows a slight loss for dogs 23, 24 and 26. Dog 25 exhibited a slight gain in weight. The alterations in body weight are, however, too small to be of any special significance.

These animals maintained upon a relatively large intake of boiled peas had far outlived the limit set by the animals in our previous work. The question therefore presented itself whether the increased period of time without symptoms could be accounted for by the larger intake of peas. To answer this query the following plan was adopted. Two of the animals, dogs 23 and 25, were maintained with the dietary unchanged. The other two, dogs 24 and 26, were fed with a similar mixture but the quantities of peas were much decreased and the oil was significantly increased. These altered dietaries follow:

Dog 24

	NITROGEN	CALORIES
	grams	
100 grams dried peas	4.30	364
70 grams cracker meal	1.32	269
45 grams cotton seed oil	0.00	418
	5.62	1051

or 0.5 gram N and 93 calories per kilo body weight

Dog 26

172 grams dried peas	7.40	626
120 grams cracker meal	2.25	478
78 grams cotton seed oil	0.00	724
	9.65	1828

or 0.5 gram N and 94 calories per kilo body weight

Up to March 24 all the dogs were in excellent condition, the food being completely eaten in each instance and the body weight being maintained. The body weights on March 26 were as follows:

DOG	MARCH 26	ORIGINAL WEIGHT
	kilos	*kilos*
23	15.8	16.2
24	11.3	11.5
25	11.8	11.4
26	19.5	20.0

On March 28 dog 24 exhibited little appetite and from this date showed less and less inclination to eat. Examination revealed nothing abnormal until March 31, when the mouth of the dog presented the characteristic pathological condition observed in our previous work. The fecal discharges were bloody and diarrhoeal in character. It is of interest to note the record of the body weight.

Body weights of dog 24

	kilos
March 26	11.3
March 28	10.9
March 30	10.0
April 1	9.1

The dog was found dead on April 1 and an autopsy was performed while the body was still warm.

Autopsy of dog 24. The mucous membrane of the mouth was exceedingly foul in odor, was greatly ulcerated and could be easily stripped off. The tongue presented a similar aspect. The teeth were firm and normal. The heart was enormously dilated and in the right ventricle were found six thin worms (Filaria), each about one foot in length. The lungs appeared to be normal. In the stomach and intestine large numbers of worms of the same type as those found in the heart were present. The stomach was normal. Throughout the small intestine were well defined areas which were distinctly hemorrhagic. The large intestine and rectum presented an extreme picture of hemorrhagic areas of the same type. The skin was normal. The joints of the legs exhibited no hemorrhages or hyperemia. The thyroids, liver, spleen, kidneys, adrenals, bladder, pancreas and brain appeared to be normal. All the principal tissues and organs were preserved in Zenkers and Orth's fluids and in 5 per cent formalin and were sent to Prof. Theobald Smith for histological examination. In

his report Professor Smith states, "there is a marked fatty degeneration of the convoluted tubules of the kidney." There were apparently no noticeable changes in the character of the intestinal epithelium.

The condition of the other three dogs remained normal until April 2 when dog 26 exhibited evidences of a diminished appetite. From this date until death never more than three-quarters of the food was eaten. Usually one-quarter to two-thirds of the diet was refused. No other symptoms were noted until the appearance of bloody stools on April 8. At the same time examination of the mouth showed a distinct inflammation of the gums and on the following day the inner surfaces of the lips were covered with pustules. The animal died on the afternoon of April 10. The body weight had remained constant until April 1. The record of body weight follows:

Body weights of dog 26

	kilos
April 1	19.3
April 3	18.9
April 5	18.1
April 7	17.7
April 9	16.6
April 10	16.1

Autopsy of dog 26. The body presented a normal appearance in general and was very well supplied with fat. All the organs seemed normal, with the exception of the gastro-enteric tract. Here the typical foul condition of the mouth was again in evidence and in the small intestine definite hemorrhagic areas were present, together with a few small tape worms. Skin and bones seemed normal. Sections from all the principal tissues and organs were preserved in Zenkers and Orth's fluids and in 5 per cent formalin and were sent to Prof. Theobald Smith for histological examination. In his report Professor Smith states, "the only definite histological changes which I can discover are a peculiar atrophy of the epithelium of the large intestine, due probably to the change in the character of the mucous secretion."

The two dogs on a higher intake of peas were maintained in excellent condition until April 15, when it was observed that each day dog 23 refused a portion of the diet. At the same time the body weight began to fall gradually. No definite symptoms of any sort could be detected. The diminished appetite continued without other noticeable symptoms until April 27, when yellowish diarrhoeal stools were observed. These persisted until death. The dog now refused all food and lay quietly in the pen, and when aroused exhibited great weakness. The mouth presented the typical foul character but was

less marked than in previous instances. On the 29th of April the animal was found dead. The body weights follow:

Body weights of dog 23

	kilos
April 12	16.0
April 15	15.9
April 19	15.7
April 21	15.5
April 23	15.1
April 25	14.7
April 27	14.1
April 29	13.6

Autopsy of dog 23. All organs and tissues appeared normal except the gastro-enteric tract. In the oesophagus, small and large intestine, the characteristic hemorrhagic condition seen in the other animals was noted. The skin, bones and teeth were normal. A considerable number of small tape worms was found in the small intestine. No tissues were preserved.

Dog 25, the companion of dog 23, gained steadily in body weight until May 1, the weight at this time being 13.1 kilos. From May 7 this animal showed evidences of decreased appetite, amounts of food varying from one-quarter to three-quarters of the total dietary being refused. At the same time the body weight gradually fell. Diarrhoeal stools were noted on May 12, otherwise the dog appeared to be normal. Food was refused on May 15, diarrhoea was prominent and the animal exhibited general weakness. This condition continued until May 17, when mucus continually dripped from the mouth. Examination revealed the typical mouth picture seen previously in other dogs. On this date the animal was unable to stand, presumably through weakness. No significant change in symptoms was observed up to the time of death on May 18.

The record of body weight follows:

Body weights of dog 25

	kilos
May 1	13.1
May 3	13.1
May 5	13.1
May 7	13.1
May 9	13.0
May 11	12.7
May 13	12.2
May 15	11.6
May 17	11.0

Autopsy of dog 25. As usual the mouth was ulcerated and foul. The typical hemorrhagic condition of the intestines and rectum was also present. Worms were absent from the gastro-enteric tract. The skin, bones and teeth as well as all the principal organs presented a normal appearance.

Summary

Four dogs maintained upon a relatively large intake of boiled peas as the principal nitrogenous source lived far beyond the limit of time set by previous animals whose diet contained a smaller quantity of boiled peas.

Two of these animals that had lived longer than this limit were fed upon a diet containing a smaller quantity of boiled peas. These animals developed symptoms and died much sooner than the two dogs that continued upon the higher intake of boiled peas. It is therefore apparent that the intake of a large quantity of peas is less detrimental to dogs than smaller quantities. If one assumes that the untoward symptoms are induced by a lack of some essential substance or substances, the so-called "vitamines," the results obtained may be interpreted to indicate that boiled peas contain a small quantity of these essential substances, too small indeed to maintain life for prolonged periods when the intake of boiled peas is below a certain point. On the other hand, life may be prolonged greatly when larger quantities of boiled peas, hence also larger amounts of substances of the "vitamine" class, are ingested.

A possible explanation for the pathological symptoms is also suggested by the common experience of finding various types of parasitic worms in the gastro-intestinal tract. That there is little or no relation between the presence of these parasites and the symptoms observed is indicated by the fact that of the four dogs just considered, three contained parasitic worms and one was free from them, and yet in all four animals the same type of symptoms appeared.

THE QUESTION OF HIGH VERSUS LOW NITROGEN INTAKE IN THE PRODUCTION OF THE DISEASED CONDITION IN DOGS

From the experience just recited concerning the influence of large and small quantities of boiled peas upon the production of the pathological symptoms in dogs, one may readily conclude that the question of nitrogen intake *per se* bears no relation whatsoever to the induction of the abnormal nutritive condition. For whether the nitrogen con-

tent of the diet containing boiled peas is large or small, the diseased condition ultimately makes its appearance. Nevertheless, in order that emphasis may be laid upon the total independence of the nitrogen content of the diet and diminished "vitamine" intake, the problem has been investigated from a different standpoint.

Two dogs, dogs 27 and 28, in splendid nutritive condition were selected. Dog 27 was a mongrel collie bitch weighing 19.3 kilos and dog 28 was a Boston bull bitch of 10.0 kilos body weight. These animals were placed upon the following diets:

Dog 27

	NITROGEN	CALORIES
	grams	
172 grams dried peas	7.40	626
120 grams cracker meal	2.25	478
78 grams cotton seed oil	0.00	724
	9.65	1828

or 0.5 gram N and 94 calories per kilo body weight

Dog 28

80 grams dried peas	3.44	291
21 grams casein (Hammarsten)	2.53	86
87 grams cracker meal	1.57	346
19 grams cotton seed oil	0.00	177
	7.54	900

or 0.75 gram N and 90 calories per kilo body weight

A study of the dietaries shows that they are so constructed as to indicate the kind of factor responsible for the death of our previous animals. One dietary, that of dog 27, may be regarded as a relatively low nitrogen diet containing probably some, although possibly a minimum amount of "vitamine" substances. The diet of dog 28, on the other hand, must be regarded as containing a sufficiency of nitrogen. In this dietary a portion of the peas which presumably contains the "vitamine" fraction was in part replaced by casein. Casein was selected because of the probability that any so-called "vitamine" substances which the protein may have originally contained would be lost in its purification.

If the reasoning which led to the institution of these experiments is correct, one might expect one of two results as the outcome of the experiment. In the first place if the death of our previous animals is related to the intake of nitrogen *per se*, dog 27 should succumb rapidly. On the other hand, under the same hypothesis, dog 28 should be maintained in good health indefinitely. Secondly, if the view is correct that the untoward symptoms are produced by a deficiency of an essential constituent presumably contained in peas, it is evident that the dog receiving the smaller quantity of peas should die first in spite of the larger nitrogen intake.

The experiment was begun on April 12, 1913. Dog 28 maintained a good nutritive condition until May 31, when all food was refused. For the next few days only one-quarter to one-half the diet was eaten. Body weight began to fall and the animal was quiet and apathetic. The characteristic foul mouth appeared as did diarrhoea, which, however, was not as prominent a feature as usual. The dog died on June 14.

The record of the body weight follows:

Body weights of dog 28

	kilos
April 12	10.0
May 29	10.3
May 31	9.9
June 2	9.6
June 4	9.4
June 6	9.1
June 8	8.9
June 10	8.9
June 12	8.7
June 14	8.4

Autopsy of dog 28. The mouth and intestinal features observed in previous animals were present. A few ascaris worms were found. Other organs and tissues seemed normal. No tissues were preserved.

The course of dog 27 presents peculiar features because of the long period of time the animal lived in a diseased condition. Up to May 1 the animal appeared normal, maintained body weight, varying between 19.4–19.2 kilos and usually ate all the food. On May 3, coincident with a decreased appetite, body weight began to fall perceptibly. The food eaten varied from one-third to one-half the diet until May 8 when all food was refused; the body weight had fallen to 18.5 kilos. On May 13 diarrhoeal stools were observed but otherwise the condition of the animal seemed normal, except that all food was refused.

Bloody stools were in evidence on May 15. On May 19 the dog seemed normal again and ate about one-half the daily food. This condition was maintained until June 3, when diarrhoea was again exhibited and lasted for two days. The body weight had gradually fallen to 14.4 kilos. The quantity of food eaten never exceeded one-half the daily ration. The animal again regained its normal appearance with the exception of appetite and body weight, and maintained it until June 30 when the characteristic mouth symptoms were observed. With the idea in mind that the animal might be brought back to her normal condition, approximately two-thirds of the nitrogen of the peas was replaced by a commercial milk powder, the other ingredients of the diet remaining unchanged. The new diet was fed on July 1. In spite of the fact that the diet was fairly well consumed, body weight continued to fall steadily and the symptoms, foul mouth and bloody diarrhoea, grew more and more serious until death occurred on July 13.

The record of body weight follows:

Body weights of dog 27

	kilos		*kilos*		*kilos*
May 1	19.2	May 27	15.9	June 22	12.8
May 3	19.0	May 29	15.6	June 24	12.7
May 5	18.9	May 31	15.1	June 26	12.4
May 7	18.8	June 2	14.8	June 28	12.1
May 9	18.5	June 4	14.4	June 30	12.0
May 11	17.8	June 6	14.1	July 2	12.1
May 13	17.3	June 8	13.9	July 4	11.9
May 15	17.0	June 10	13.9	July 6	11.4
May 17	16.7	June 12	13.7	July 8	11.0
May 19	16.6	June 14	13.5	July 10	10.4
May 21	16.4	June 16	13.3	July 12	9.9
May 23	16.1	June 18	13.0	July 14	9.9
May 25	16.0	June 20	12.8		

Autopsy of dog 27. The entire enteric tract presented a striking hemorrhagic appearance. The mouth was foul and ulcerated. All other organs and tissues seemed normal.

It will be remembered that dog 28 was receiving a relatively high nitrogen intake and by the rapid death of this animal under the experimental conditions it must be concluded that *the level of nitrogen intake of itself has little or nothing to do with the result obtained.* This is emphasized by the fact that dog 27, maintained on a lower nitrogen level, did not succumb as soon as dog 28. It is also apparent that *the diet containing boiled peas furnished more of an unknown*

substance or substances essential for life than did the dietary in which part of the boiled peas was replaced by casein.

If further evidence is needed to prove that the level of nitrogen intake plays little or no rôle in the production of the condition under discussion, it may be furnished by the experience with dog 21, page 22 and dog 17, page 26, animals that developed the characteristic symptoms when fed upon a diet containing a relatively small intake of boiled peas, hence low in nitrogen. After placing the dogs upon a meat diet the abnormal symptoms disappeared. Later these dogs were given approximately the same nitrogen intake as when upon the diet of boiled peas and they lived until the conclusion of the investigation without any manifestations of malnutrition. In the case of dog 21, the experiment on low nitrogen with meat in the diet continued for more than four months. With dog 17 normal conditions were maintained for more than six months.

THE DEVELOPMENT OF THE DISEASED CONDITION IN DOGS MAINTAINED ON DIETS CONTAINING MEAT

During the course of an investigation in which the meat of the diet was significantly decreased at intervals, the pathological symptoms characteristic for dogs fed upon food containing boiled peas made their appearance. In some of these cases the symptoms observed were apparently quite distinct from those previously noted. These unusual appearances were closely associated with an influence upon the nervous system as indicated by severe convulsions. That this symptom is, however, closely related to the pathological condition characteristic of a diet containing boiled peas is rendered probable by the fact that in one animal both types of symptoms were in evidence, that is, convulsions and the peculiar sore mouth were both present.

In the experiments to be outlined, balance periods of ten days each were carried through at intervals of every twenty days, so that from the standpoint of nitrogen equilibrium data are at hand to furnish conclusions concerning the nutritive condition of the animals. For lack of space these data in detail are omitted from the present report. Inasmuch as all the dogs in this group were maintained upon the same types of diet at corresponding intervals, these data in detail are recorded only with dog 1. The experience with this group of dogs is given below.

Dog 1. This animal, obtained on July 6, 1905, was a brown, short haired mongrel in fine nutritive condition weighing 10.2 kilos. She was young but fullgrown.

From July 6 to July 18 a constant diet was given consisting of meat, cracker meal and lard, containing 9.16 grams nitrogen and approximately 813 calories, or 0.90 gram nitrogen and 80 calories per kilo body weight. On this diet the body weight increased to 10.6 kilos and a positive nitrogen balance was obtained. On July 30 the diet was changed to 110 grams meat, 80 grams cracker meal and 46 grams lard. The new diet contained 5.56 grams nitrogen and about 888 calories, or approximately 0.52 gram nitrogen and 83 calories per kilo. With this diet a slight negative nitrogen balance was obtained. The food intake remained unchanged until August 25, when the meat of the diet was still further reduced. The new dietary consisted of 50 grams meat, 90 grams cracker meal and 50 grams lard, containing a total of 3.39 grams nitrogen and approximately 888 calories, or 0.30 gram nitrogen and 78 calories per kilo. On this diet a slight negative nitrogen balance was obtained.

On October 18 the diet was so changed that in the morning meal the dog received 120 grams milk, 62 grams cracker meal and 11 grams lard. The afternoon meal consisted of 25 grams meat, 45 grams cracker meal and 25 grams of lard. The alteration in the character of the dietary did not change the intake of nitrogen nor the fuel value. On November 3 the nitrogen intake was diminished by the ingestion of the following food: For the morning meal 88 grams milk, 62 grams cracker meal and 14 grams lard; for the afternoon meal 25 grams meat, 39 grams cracker meal and 28 grams lard. The total nitrogen and calorific intake amounted to 3.05 grams and 897 calories respectively, or 0.28 gram nitrogen and 80 calories per kilo body weight. The dietary was again changed on November 22, so that the dog received 50 grams meat, 92 grams of bread and 50 grams of lard. The nitrogen intake remained constant, although there was a slight reduction in the fuel value. On this diet there was a slight negative nitrogen balance.

On December 2 the food intake consisted of 88 grams milk, 62 grams cracker meal and 14 grams lard for the morning meal; in the afternoon meal were contained 25 grams meat, 39 grams cracker meal and 28 grams lard. The nitrogen intake was not altered. This diet was maintained until December 6 when boiled rice was substituted for the cracker meal, 125 grams of rice replacing 101 grams cracker meal. The total nitrogen of the new diet amounted to 2.64 grams and the fuel value was 816 calories, or 0.26 gram nitrogen and 80 calories per kilo body weight. On this intake of food the dog was found to be in almost perfect nitrogen balance.

The diet was changed on February 9 to 50 grams meat, 76 grams cracker meal, and 46 grams lard, yielding 3.04 grams nitrogen and 740 calories per day, or 0.29 gram nitrogen and 81 calories per kilo body weight. This diet was maintained until February 24, when it was changed to the following: 48 grams meat, 128 grams rice and 30 grams lard. It contained 3.04 grams nitrogen and 818 calories, or 0.30 gram nitrogen and 81 calories per kilo body weight. The animal at this time weighed 10.1 kilos.

On the afternoon of February 28 the dog vomited a portion of the food which was greedily eaten again but was subsequently regurgitated. Nothing abnormal could be noted in the animal's appearance or behavior. On March 1 the dog refused to eat rice so the diet was changed to 48 grams meat, 76 grams cracker meal and 46 grams lard, leaving the nitrogen intake unaltered but diminishing

the fuel value to 740 calories, or 73 calories per kilo. The next day the bitch would not touch this diet so was offered 100 grams meat containing 3.63 grams nitrogen, which was greedily devoured. The following day the food intake was as follows: 100 grams meat, 76 grams cracker meal and 46 grams lard, yielding a total nitrogen content of 4.93 grams with a fuel value of 798 calories, or 0.48 gram nitrogen and 79 calories per kilo. At this time the dog seemed normal. About 11 o'clock on the morning of March 5 the animal was taken with a seizure resembling that of epilepsy. After application of cold water to the head and warmth to the hind extremities the normal condition was soon resumed. Food was eaten with apparent relish and no further untoward symptoms were observed.

The food was changed on February 9 to 60 grams meat, 76 grams cracker meal and 46 grams lard, containing 3.47 grams nitrogen and 750 calories, or 0.34 gram nitrogen and 77 calories per kilo body weight. This diet was maintained until March 22. On this date about 9 a.m., the dog was suddenly taken with a seizure resembling that of March 5. Cold applications to the head caused a resumption of normal conditions for a short period only. At intervals of five or six minutes convulsions ensued, which were abated somewhat by the cold applications. In the period between these seizures efforts were made to walk around the room. This the dog succeeded in accomplishing in a blind, staggering manner. Apparently she was partially blind, for she would run her nose into a wall as though she did not see it. The left eye was closed and twitching and the left legs did not respond to her will, for in walking the toes would be doubled under the ball of the foot, not touching the floor. The right side seemed normal. After a large number of convulsions no effort was made to rise. At this stage she seemed greatly fatigued. During the latter part of the afternoon the convulsions occurred at shorter inervals but their duration was much less. At periods extreme muscular relaxation was present, at others extreme rigor. During the seizures the pupils of the eyes were greatly dilated and the nose very warm; between these intervals the pupils were much contracted. At 10.30 p.m. inspection showed that the dog had vomited and defecated. The feces were normal in appearance. A copious hemorrhage, presumably through the nose, had also occurred. Instead of well marked convulsions, she lay in a state of what appeared to be a mild continuous spasm. This condition was unchanged until 10 a.m., March 23, when the dog died without extraordinary movements or behavior; respiration gradually ceased.

Autopsy of dog 1. Large masses of subcutaneous fat and much fat around the kidneys were present. At the pyloric end of the duodenum there was a spot about the size of a silver dollar which had a peculiar yellowish-red appearance. In the center of this spot the intestine was practically perforated and bile had penetrated between the muscular and connective tissue layers lending to the area the peculiar color. A similar but much larger area was observed at the beginning of the rectum. Comparison with the apparently normal ileum demonstrated that the remainder of the intestine was extremely thin. In fact, with the exception of the ileum, the entire intestine seemed to

consist of little more than the connective tissue covering. On the liver were found peculiar yellow spots. Upon section of the kidneys a pus-like fluid exuded on squeezing. The spleen was normal. The right lung was normal but the left was congested. Heart, brain, mouth and oesophagus were normal. Cultures were made from the various organs and of the blood but the results were negative.

Dog 2 was a black long haired bitch weighing 10.7 kilos, in excellent nutritive condition. Although young, she was full grown and had borne young. She was maintained upon the same types of food in similar quantities in accordance with her body weight as obtained for dog 1 and at corresponding periods. In general, the animal remained in nitrogen equilibrium. No symptoms of any significance were observed from the beginning of the experiment on July 6 up to February 25. On this day the dog failed to eat all her food but careful examination revealed nothing abnormal. Worms (ascaris) were found in the stools of February 26. The animal did not eat well and vomited whatever was eaten. The anus appeared to be in a state of irritation, judging by her behavior. She was restless and appeared in pain. Vermifuge was administered but was vomited immediately. The next morning the animal was found dead.

At autopsy the large quantities of fat around the kidneys and that deposited subcutaneously were very striking. All organs appeared normal, except the intestine, which contained peculiar spots or depressions (ulcers).

Dog 11. This animal was a fullgrown Airedale terrier of 10.7 kilos, in fine nutritive condition. Although she was apparently about two years of age there was no evidence that young had ever been borne. The observations were begun on September 14. The course of the experiment was uneventful until February 4, when at 6 a.m. the dog went into convulsions. Cold water applied to the head and heat to the extremities soon resulted in a resumption of normal conditions. No abnormalities of any sort could be discovered on this day, but on February 5 examination of the mouth revealed the typical appearance seen in the case of dogs fed on boiled peas. Although these pathological symptoms were in evidence, the dog ate greedily. The stools on this day were diarrhoeal in character and contained blood. On February 7 the animal was in a condition resembling "palsy" and one convulsion occurred. No food was taken and the stools were bloody and diarrhoeal. The animal ran back and forth in her pen as though in pain, and the hind legs appeared to be very weak. An improvement was apparent on February 8, as the palsy-like symptoms had disappeared. No food was eaten. The mouth was putrid in odor and mucous strings hung from it. The following day the animal seemed stronger and drank milk and ate meat. On February 10 the animal would not eat and lay quietly in the pen. Death occurred on February 11. Just before death the dog lay in a comatose condition. During the course of the experiment body weight had risen from 10.7 kilos to 11.5 kilos and nitrogen equilibrium was usually maintained.

On autopsy it was found that the entire mucous membrane of the mouth was absent. The duodenum contained ulcerated spots with distinct areas of congestion around the ulcers. The small intestine was but slightly congested, whereas the large intestine was a mass of bloody tissue.

Dog 12 was a Boston bull bitch of 12.7 kilos, in excellent condition. The experiment was begun on September 14 and was uneventful up to May 28, at which time the bitch was in nitrogen balance. At this period there seemed to be a loss of appetite. She was immediately placed upon a strictly meat diet without altering the nitrogen intake. Vermifuge was administered but no worms were passed. The following day it was discovered that the mouth was sore. The feces were diarrhoeal, but contained no blood. On May 29 all food was refused and the dog lay quietly in the pen. The condition of the mouth grew worse. Death occurred on June 1.

On post mortem examination it was found that all the organs and tissues were apparently normal except that in the intestine definite spots occurred indicating ulceration. The mouth was characteristically foul and almost devoid of mucous membrane.

An autopsy revealed nothing abnormal.

Dog 14 was a black retriever bitch of 12.8 kilos, in fine condition. The experiment was begun on September 14 and until November 18 nothing of significance was observed. On this date the dog was seized with violent convulsions. The usual remedies were applied and the convulsions ceased, only to be followed by a period of coma, which a few hours later terminated in death.

Summary

Five dogs maintained in nitrogenous equilibrium upon diets containing some meat succumbed in periods of time varying from two to eight months. In four animals convulsions were in evidence. In two of these instances ulcers of the duodenum were the only signs of abnormality visible upon autopsy. In a third, no pathological tissues were noted. The fourth dog showed the convulsions to be associated with the condition of foul mouth, characteristic for dogs fed upon diets containing boiled peas. The fifth animal died without convulsions but exhibited the symptoms usual with dogs fed upon boiled peas. From these facts it is probable that the convulsions and symptoms of sore mouth, etc., are induced by the same set of nutritive conditions.

In every case in this group the dogs showed excellent utilization of the food.

The dietary always included meat but was so constructed that the total nitrogen intake was relatively small, at the time of death being for dog 1, 0.34 gram of nitrogen per kilo of body weight; for dog 2 an equivalent of 0.30 gram; 0.25 gram for dog 11; 0.34 gram for dogs 12 and 14.

The results obtained are interpreted to indicate that even though meat may be continually present in the diet, reduction of the quantity to an undefined limit may cause characteristic pathological symptoms to appear, eventually leading to death. From our experience with animals upon a diet containing boiled peas, there seems no occasion for associating the symptoms observed in these experiments with the relatively low nitrogen intake *per se*.

GENERAL SUMMARY AND CONCLUSIONS

Dogs fed upon a diet consisting of boiled (dried) peas, cracker meal, cotton seed oil or lard, rapidly develop symptoms indicating abnormal nutrition. This condition eventually terminates in death.

Previous to the development of the pathological manifestations, the dogs are usually in nitrogen balance and exhibit excellent food utilization. The nitrogen partition of the urine is normal when compared to that of animals maintained upon the same level of nitrogen intake.

The pathological symptoms at times can be made to disappear and the normal conditions of nutritive rhythm can be reëstablished by addition of meat to the dietary.

In the production of the symptoms, it is immaterial whether the transition from a diet containing meat to one of vegetable origin is sudden or gradual. The final outcome is the same in both cases.

The intake of a large quantity of peas is less detrimental than smaller amounts.

In the development of the pathological condition, the level of nitrogen intake as such plays little or no rôle.

The typical symptoms may be induced in dogs, but with much greater difficulty when a diet containing meat, cracker meal and lard is fed in appropriate quantities. For the production of the diseased condition the meat intake must be reduced to a certain undefined minimum. Under these circumstances less than fifty per cent of dogs exhibit pathological symptoms and these may appear in periods of two to eight months.

From the facts enumerated the conclusion seems tenable that the abnormal state may be referred to a deficiency of some essential

dietary constituent or constituents, presumably belonging to the group of hitherto unrecognized but essential components of an adequate diet.

In the essential features, the pathological manifestations described in this investigation closely resemble those which may be observed in human pellagra.

Bitch 6, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE*							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
August 2	5.6	4.22	140	1.053	3.20	2.68	0.090	0.065	0.62		
August 3	5.6	4.22	120	1.031	3.24	2.72	0.133	0.065	0.61	11.0	
August 4	5.6	4.22	130	1.025	3.35	2.89	0.097	0.065	0.53	44.0	
August 5	5.6	4.22	130	1.026	3.38	3.06	0.137	0.065	0.68	30.5	
August 6	5.6	4.22	150	1.022	3.53	3.14	0.137	0.066	0.61	24.0	
August 7	5.6	4.22	120	1.038	3.13	2.73	0.110	0.054	0.59	30.0	
August 8	5.6	4.22	200	1.025	3.35	3.00	0.202	0.069	0.65	36.5	
August 9	5.6	4.22	130	1.036	2.99	2.11	0.158	0.051	0.63	32.0	
August 10	5.6	4.22	240	1.014	3.49	3.11	0.166	0.051	0.67	21.0	
August 11	5.6	4.22	110	1.036	3.02	2.58	0.120	0.065	0.63	69.5	
Total		42.20			32.68					298.5	7.52
Average per day		4.22	197	1.031	3.27	2.80	0.135	0.062	0.62		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
42.20	Through the urine	32.68
	Through the faeces	7.52
	Through the hair (12.5 grams)	1.46
42.20		41.66

Nitrogen balance for ten days = + 0.54 *grams*

Nitrogen balance per day = + 0.054

Intake per day 0.75 gram nitrogen and 96.6 calories per kilo of body-weight

*Unless otherwise noted the reaction of the urine was acid to litmus.

Bitch 7, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE*							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
August 2.........	9.3	7.00	140	1.045	4.43	3.73	0.291	0.094	0.68	17.0	
August 3.........	9.0	7.00	100	1.045	4.36	3.70	0.331	0.091	0.58	44.0	
August 4.........	9.0	7.00	100	1.040	4.21	4.92	0.256	0.093	0.46	63.0	
August 5.........	9.0	7.00	80	1.035	3.10	2.66	0.292	0.089	0.60	115.0	
Total.................		28.00			16.10					239.0	7.74
Average per day.......		7.00	105	1.041	4.03	3.75	0.292	0.092	0.58		

Nitrogen balance—four days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
28.00	Through the urine................	16.10
	Through the faeces...............	7.74
	Through the hair (3.5 grams)......	0.38
28.00		24.22

grams

Nitrogen balance for four days = + 3.78

Nitrogen balance per day = + 0.38

Intake per day 0.75 gram nitrogen and 96.0 calories per kilo of body-weight

Animal developed worms and period stopped.

Bitch 8, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
August 2	11.2	8.44	200	1.034	6.59	5.59	0.313	0.143	1.00	41.0	
August 3	11.2	8.44	220	1.027	6.41	5.75	0.256	0.148	0.96		
August 4	11.2	8.44	180	1.031	6.26	5.25	0.263	0.147	0.81	65.5	
August 5	11.2	8.44	140	1.042	6.48	5.76	0.292	0.145	0.86	77.0	
August 6	11.2	8.44	140	1.040	6.66	4.76	0.347	0.141	1.42	62.0	
August 7	11.2	8.44	170	1.045	5.90	5.18	0.331	0.143	0.93	66.5	
August 8	11.2	8.44	290	1.024	6.62	6.05	0.328	0.148	0.96	89.5	
August 9	11.4	8.44	250	1.026	6.66	5.85	0.407	0.138	1.09	38.0	
August 10	11.4	8.44	390	1.017	6.91	6.06	0.346	0.132	0.67	72.0	
August 11	11.4	8.44	140	1.041	5.87	5.18	0.288	0.138	0.93	117.0	
Total		84.40			64.36					628.5	18.30
Average per day		8.44	212	1.033	6.44	5.54	0.317	0.142	0.86		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
84.40	Through the urine	64.36
	Through the faeces	18.30
	Through the hair (12.7 grams)	1.63
84.40		84.29

Nitrogen balance for ten days = + 0.11 *gram*

Nitrogen balance per day = + 0.011

Intake per day 0.75 gram nitrogen and 96.6 calories per kilo of body-weight.

Bitch 9, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
August 2	12.0	9.03	220	1.031	5.90	4.90	0.251	0.145	1.27		
August 3	12.2	9.03	290	1.025	6.88	5.28	0.306	0.155	1.06	37.5	
August 4	12.4	9.03	160	1.040	6.30	5.34	0.168	0.152	0.98	47.5	
August 5	12.4	9.03	220	1.034	5.98	5.25	0.219	0.154	1.01	73.0	
August 6	12.4	9.03	100	1.055	5.47	4.87	0.133	0.145	0.85	44.0	
August 7	12.4	9.03	190	1.030	6.23	5.46	0.259	0.152	0.85	85.5	
August 8	12.4	9.03	200	1.035	6.34	5.54	0.331	0.164	1.05	106.0	
August 9	12.4	9.03	210	1.035	6.80	5.94	0.241	0.154	1.18	25.0	
August 10	12.4	9.03	440	1.016	7.13	5.08	0.313	0.145	1.03	62.0	
August 11	12.4	9.03	270	1.035	6.05	5.22	0.176	0.147	1.14	116.5	
Total		90.30			63.08					597.0	18.27
Average per day		9.03	230	1.034	6.31	5.29	0.239	0.151	1.04		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
90.30	Through the urine	63.08
	Through the faeces	18.27
	Through the hair (31.5 grams)	4.47
90.30		85.82

Nitrogen balance for ten days = + 4.48 *grams*
Nitrogen balance per day = + 0.45

Intake per day 0.75 gram nitrogen and 96.0 calories per kilo of body-weight.

Bitch 10, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
August 2	14.4	10.95	265	1.036	7.38	6.00	0.295	0.164	1.43		
August 3	14.4	10.95	185	1.038	6.84	5.24	0.331	0.174	1.41	43.5	
August 4	14.6	10.95	170	1.035	5.72	4.81	0.241	0.176	0.84	64.0	
August 5	14.2	10.95	190	1.046	7.67	6.49	0.241	0.182	1.43	96.0	
August 6	14.4	10.95	120	1.035	5.62	5.10	0.234	0.179	1.10	90.5	
August 7	14.2	10.95	160	1.042	7.45	6.46	0.385	0.179	1.30	46.0	
August 8	14.2	10.95	230	1.030	7.42	6.57	0.378	0.193	1.35	108.0	
August 9	14.4	10.95	240	1.042	9.25	8.07	0.533	0.176	1.48	39.0	
August 10	14.4	10.95	270	1.033	9.00	7.56	0.504	0.152	1.41	126.0	
August 11	14.4	10.95	300	1.032	7.34	6.10	0.630	0.152	1.49	207.0	
Total		109.50			73.69					820.0	25.58
Average per day		10.95	213	1.037	7.37	6.24	0.377	0.173	1.32		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
109.50	Through the urine	73.69
	Through the faeces	25.58
	Through the hair (61.5 grams)	7.40
109.50		106.67

Nitrogen balance for ten days = + 2.83 *grams*
Nitrogen balance per day = + 0.28

Intake per day 0.76 gram nitrogen and 97.4 calories per kilo of body-weight.

Bitch 18, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
October 22	13.1	6.81	660	1.008	4.70	3.84	0.380	0.225	0.67		
October 23	13.1	6.81	570	1.010	4.66	3.83	0.290	0.201	0.63		
October 24	13.2	6.81	680	1.008	4.65	3.84	0.290	0.173	0.65	40.0	
October 25	13.2	6.81	680	1.010	4.80	4.09	0.280	0.163	1.04	25.0	
October 26	13.2	6.81	510	1.013	4.82	4.17	0.260	0.158	0.86	27.0	
October 27	13.3	6.81	490	1.012	4.68	3.95	0.280	0.159	0.97	25.0	
October 28	13.3	6.81	470	1.015	4.57	4.09	0.225	0.164	1.02	25.0	
October 29	13.3	6.81	590	1.010	4.96	4.31	0.310	0.155	1.04	19.0	
October 30	13.3	6.81	630	1.010	4.87	3.96	0.280	0.154	1.08	27.0	
October 31	13.3	6.81	610	1.010	4.24	3.82	0.250	0.155	1.13	111.0	
Total		68.10			46.95					299.0	12.20
Average per day		6.81	589	1.010	4.69	3.99	0.284	0.170	0.90		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
68.10	Through the urine	46.95
	Through the faeces	12.20
	Through the hair (31.9 grams)	3.55
68.10		62.70

Nitrogen balance for ten days = + 5.40 *grams*

Nitrogen balance per day = + 0.54

Intake per day 0.51 gram nitrogen and 98 calories per kilo of body-weight.

Bitch 18, period 2

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
December 6.....	14.0	3.95	730	1.007	2.64	2.14	0.260	0.120	0.62		
December 7.....	14.2	3.95	960	1.007	3.30	2.49	0.390	0.143	0.74		
December 8.....	14.4	3.95	565	1.010	3.53	2.64	0.340	0.146	0.77		
December 9.....	14.3	3.95	890	1.007	3.30	2.83	0.340	0.138	0.91	11.0	
December 10.....	14.4	3.95	700	1.009	3.26	2.75	0.330	0.140	0.74		
December 11.....	14.4	3.95	860	1.017	3.54	2.72	0.340	0.145	0.79		
December 12.....	14.5	3.95	950	1.007	3.30	2.63	0.340	0.136	0.99		
December 13.....	14.6	3.95	900	1.007	3.06	2.53	0.350	0.150	0.99	39.0	
December 14.....	14.4	3.95	840	1.007	3.35	2.68	0.340	0.145	1.02	23.0	
December 15.....	14.5	3.95	680	1.010	2.98	2.62	0.360	0.130	0.75	14.0	
Total..................		39.50			32.20					87.0	3.42
Average per day.......		3.95	807	1.008	3.20	2.60	0.339	0.139	0.83		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
39.50	Through the urine...............	32.20
	Through the faeces...............	3.42
	Through the hair (13.0 grams)....	1.47
39.50		37.09

Nitrogen balance for ten days = + 2.41 *grams*

Nitrogen balance per day = + 0.24

Intake per day 0.27 gram nitrogen and 72.0 calories per kilo of body-weight.

Bitch 21, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
January 16	13.6	4.76	560	1.008	3.61	2.97	0.350	0.151	0.71		
January 17	13.7	4.76	520	1.011	3.92	3.40	0.310	0.162	0.64	47.0	
January 18	13.7	4.76	640	1.010	3.86	3.24	0.370	0.159	0.70		
January 19	13.8	4.76	580	1.010	3.83	3.17	0.340	0.160	0.69		
January 20	13.8	4.76	580	1.009	3.44	2.89	0.340	0.159	0.69	38.0	
January 21	13.8	4.76	590	1.008	2.81	2.47	0.270	0.101	0.52		
January 22	13.9	4.76	830	1.010	4.86	3.92	0.510	0.198	0.83	37.0	
January 23	13.9	4.76	470	1.010	3.64	2.97	0.270	0.152	0.69	35.0	
January 24	13.9	4.76	750	1.008	3.83	3.08	0.380	0.141	0.82	38.0	
January 25	13.9	4.76	510	1.010	3.53	2.77	0.250	0.141	0.72	51.0	
Total		47.60			37.33					246.0	8.41
Average per day		4.76	603	1.009	3.73	3.09	0.340	0.152	0.71		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
47.60	Through the urine	37.33
	Through the faeces	8.41
	Through the hair (13.8 grams)	1.54
47.60		47.28

Nitrogen balance for ten days = + 0.32 *gram*

Nitrogen balance per day = + 0.03

Intake per day 0.34 gram nitrogen and 89.0 calories per kilo of body-weight.

Bitch 21, period 2

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
February 13	14.1	4.20	260	1.015	3.30	2.89	0.260	0.160	0.64		
February 14	14.2	4.20	460	1.008	3.42	2.87	0.260	0.166	0.55		
February 15	14.3	4.20	340	1.013	3.69	3.10	0.260	0.163	0.67	7.0	
February 16	14.4	4.20	240	1.010	4.27	3.77	0.300	0.173	0.65		
February 17	14.3	4.20	470	1.010	4.25	3.74	0.260	0.154	0.68	24.0	
February 18	14.4	4.20	440	1.011	4.44	3.87	0.270	0.162	0.57		
February 19	14.3	4.20	320	1.015	4.38	3.76	0.260	0.157	0.64		
February 20	14.3	4.20	360	1.015	4.41	3.72	0.270	0.159	0.70		
February 21	14.3	4.20	480	1.013	4.75	4.12	0.270	0.160	0.77		
February 22	14.3	4.20	440	1.010	4.74	4.09	0.290	0.165	0.69	23.0	
Total		42.00			41.65					54.0	2.92
Average per day		4.20	381	1.012	4.16	3.59	0.270	0.162	0.66		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
42.00	Through the urine	41.65
	Through the faeces	2.92
	Through the hair (16.7 grams)	1.92
42.00		46.49

		grams
Nitrogen balance for ten days	=	− 4.49
Nitrogen balance per day	=	− 0.45

Intake per day 0.30 gram nitrogen and 76.0 calories per kilo of body-weight.

Bitch 21, period 3

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
March 13	14.4	4.80	320	1.015	4.59	3.72	0.300	0.165	0.67	15.0	
March 14	14.4	4.80	320	1.016	4.11	3.51	0.210	0.162	0.58		
March 15	14.4	4.80	340	1.017	4.47	3.49	0.260	0.180	0.67		
March 16	14.4	4.80	480	1.015	4.82	4.06	0.260	0.171	0.70		
March 17	14.3	4.80	320	1.022	4.56	3.96	0.240	0.160	0.67		
March 18	14.4	4.80	460	1.016	5.04	4.40	0.280	0.163	0.79		
March 19	14.4	4.80	240	1.025	4.92	4.25	0.260	0.161	0.69	24.0	
March 20	14.3	4.80	350	1.020	5.25	4.34	0.280	0.161	0.74		
March 21	14.2	4.80	300	1.020	5.16	4.48	0.290	0.171	0.76		
March 22	14.2	4.80	310	1.021	5.10	4.40	0.250	0.164	0.78	24.0	
Total		48.00			48.02					63.0	3.08
Average per day		4.80	344	1.019	4.80	3.66	0.263	0.166	0.70		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
48.00	Through the urine	48.02
	Through the faeces	3.08
	Through the hair (10.9 grams)	1.24
48.00		52.34

grams

Nitrogen balance for ten days = − 4.34

Nitrogen balance per day = − 0.43

Intake per day 0.33 gram nitrogen and 77.0 calories per kilo of body-weight.

Bitch 21, period 4

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
April 10..........	14.1	4.86	240	1.026	5.01	4.19	0.310	0.153	0.57	16.0	
April 11..........	14.1	4.86	150	1.045	5.70	4.74	0.360	0.155	0.73		
April 12..........	14.2	4.86	130	1.030	4.29	3.49	0.330	0.157	0.64	15.0	
April 13..........	14.1	4.86	190	1.037	4.05	3.25	0.320	0.159	0.77		
April 14..........	14.3	4.86	180	1.042	3.63	2.98	0.260	0.144	0.76	32.0	
April 15..........	14.2	4.86	270	1.022	4.17	3.38	0.370	0.145	0.80		
April 16..........	14.3	4.86	250	1.028	4.17	3.45	0.300	0.142	0.84		
April 17..........	14.3	4.86	220	1.027	3.84	3.25	0.260	0.147	0.65	22.0	
April 18..........	14.3	4.86	180	1.025	3.99	3.42	0.200	0.147	0.72		
April 19..........	14.3	4.86	240	1.030	4.14	3.37	0.260	0.155	0.61	21.0	
Total..................		48.60			42.99					106.0	4.42
Average per day........		4.86	205	1.031	4.30	3.55	0.307	0.150	0.71		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
48.60	Through the urine...............	42.99
	Through the faeces...............	4.42
	Through the hair (23.8 grams)...	2.54
48.60		49.95

grams

Nitrogen balance for ten days = − 1.35

Nitrogen balance per day = − 0.13

Intake per day 0.34 gram nitrogen and 79.0 calories per kilo of body-weight.

Bitch 21, period 5

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
May 8	14.3	4.85	1090	1.005	4.54	3.88	0.440	0.160	0.46	8.0	
May 9	14.4	4.85	1160	1.005	3.82	3.25	0.330	0.159	0.71		
May 10	14.3	4.85	1090	1.005	4.03	3.19	0.340	0.157	0.79	14.0	
May 11	14.5	4.85	1240	1.007	3.67	3.05	0.300	0.157	0.82	17.0	
May 12	14.6	4.85	1070	1.008	3.50	2.88	0.290	0.159	0.73	28.0	
May 13	14.5	4.85	800	1.010	3.51	2.91	0.280	0.162	0.64		
May 14	14.5	4.85	930	1.010	4.02	3.37	0.290	0.157	0.59	15.0	
May 15	14.6	4.85	1150	1.010	4.18	3.58	0.310	0.162	0.79		
May 16	14.6	4.85	1150	1.010	3.96	3.31	0.290	0.154	0.65	15.0	
May 17	14.5	4.85	1180	1.011	4.05	3.37	0.300	0.157	0.82	8.0	
Total		48.50			39.28					105.0	8.66
Average per day		4.85	1086	1.008	3.93	3.28	0.317	0.158	0.70		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
48.50	Through the urine	39.28
	Through the faeces	8.66
	Through the hair (19.2 grams)	1.69
48.50		49.63

Nitrogen balance for ten days = − 1.13 *grams*
Nitrogen balance per day = − 0.11

Intake per day 0.33 gram nitrogen and 80.0 calories per kilo of body-weight.

Bitch 21, period 6

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
June 5	15.4	4.85	640	1.008	3.90	3.22	0.270	0.154	0.72		
June 6	15.3	4.85	470	1.010	4.07	3.33	0.300	0.159	0.55		
June 7	15.3	4.85	500	1.010	4.32	3.68	0.280	0.133	0.53	23.0	
June 8	15.3	4.85	660	1.010	4.45	3.74	0.250	0.151	0.61		
June 9	15.4	4.85	580	1.008	4.15	3.55	0.270	0.155	0.72		
June 10	15.3	4.85	550	1.009	4.28	3.67	0.240	0.154	0.64		
June 11	15.3	4.85	500	1.010	4.36	3.70	0.260	0.152	0.71	14.0	
June 12	15.4	4.85	580	1.010	4.49	3.62	0.290	0.171	1.12		
June 13	15.3	4.85	510	1.010	4.24	3.60	0.280	0.155	0.66	21.0	
June 14	15.3	4.85	530	1.010	4.25	3.56	0.290	0.152	0.56	6.0	
Total		48.50			42.51					64.0	3.84
Average per day		4.85	552	1.009	4.25	3.57	0.273	0.154	0.68		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
48.50	Through the urine	42.51
	Through the faeces	3.84
	Through the hair (22.1 grams)	2.61
48.50		48.96

Nitrogen balance for ten days = − 0.46 *gram*
Nitrogen balance per day = − 0.05

Intake per day 0.31 gram nitrogen and 76.0 calories per kilo of body-weight.

Bitch 16, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
October 22	17.6	8.87	855	1.015	7.32	6.45	0.270	0.245	1.46		
October 23	17.6	8.87	680	1.016	7.01	6.19	0.330	0.245	1.24	18.0	
October 24	17.6	8.87	720	1.015	6.67	5.44	0.370	0.239	1.37	20.0	
October 25	17.8	8.87	770	1.015	7.02	6.05	0.265	0.214	1.62		
October 26	17.8	8.87	840	1.013	7.56	5.54	0.370	0.202	1.42	29.0	
October 27	17.8	8.87	715	1.016	7.44	6.55	0.310	0.202	1.54	29.0	
October 28	17.8	8.87	530	1.021	6.72	5.74	0.350	0.202	1.50		
October 29	17.8	8.87	740	1.015	7.06	6.27	0.350	0.148	1.47	17.0	
October 30	17.8	8.87	670	1.016	6.58	5.77	0.330	0.200	1.46	22.0	
October 31	17.9	8.87	590	1.020	6.59	5.64	0.260	0.214	1.52	28.0	
Total		88.70			69.97					163.0	9.00
Average per day		8.87	710	1.016	7.00	5.96	0.320	0.211	1.46		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
88.70	Through the urine	69.97
	Through the faeces	9.00
	Through the hair (25.8 grams)	2.94
88.70		81.91

Nitrogen balance for ten days = + 6.79 *grams*
Nitrogen balance per day = + 0.68
Intake per day 0.50 gram nitrogen and 80 calories per kilo of body-weight.

Bitch 17, period 1

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1905	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
October 22......	18.2	9.06	880	1.012	7.92	7.26	0.340*	0.215	1.54		
October 23......	18.3	9.06	800	1.015	8.15	7.56	0.680	0.242	1.62		
October 24......	18.4	9.06	685	1.017	7.63	7.01		0.243	1.49		
October 25......	18.5	9.06	930	1.012	8.22	7.80	0.430	0.247	1.68	17.0	
October 26......	18.3	9.06	770	1.015	7.72	7.40	0.430	0.218	1.43	28.0	
October 27......	18.3	9.06	770	1.015	7.83	7.34	0.400	0.221	1.62	19.0	
October 28......	18.3	9.06	710	1.016	7.51	6.97	0.430	0.220	1.55	19.0	
October 29......	18.3	9.06	700	1.015	7.10	6.62	0.380	0.202	1.20	23.0	
October 30......	18.3	9.06	770	1.016	8.05	7.34	0.650	0.220	1.53	34.0	
October 31......	18.3	9.06	570	1.021	7.14	6.51		0.233	1.46	22.0	
Total		90.60			77.27					162.0	6.66
Average per day		9.06	758	1.015	7.73	7.18	0.467	0.226	1.51		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
90.60	Through the urine	77.27
	Through the faeces	6.66
	Through the hair (23.7 grams)....	2.88
90.60		86.81

grams

Nitrogen balance for ten days = + 3.79

Nitrogen balance per day = + 0.38

Intake per day 0.49 gram nitrogen and 80.0 calories per kilo of body-weight.

*This animal had a touch of cystitis, hence the variations in the ammonia nitrogen.

Bitch 17, period 2

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
January 30......	17.4	5.77	550	1.012	3.78	3.13	0.360	0.233	0.84		
January 31......	17.5	5.77	860	1.010	3.89	2.94	0.520	0.240	0.67		
February 1......	17.4	5.77	400	1.015	3.90	2.88	0.450	0.241	0.81	26.0	
February 2......	17.5	5.77	500	1.017	4.00	3.07	0.390	0.236	0.79		
February 3......	17.6	5.77	610	1.012	4.37	3.33	0.530	0.236	0.79		
February 4......	17.6	5.77	420	1.015	4.47	3.48	0.480	0.227	0.88		
February 5......	17.6	5.77	470	1.012	4.03	3.23	0.410	0.222	0.61		
February 6......	17.7	5.77	570	1.012	3.99	3.02	0.470	0.236	0.69		
February 7......	17.7	5.77	440	1.015	4.32	3.39	0.430	0.233	0.80	47.0	
February 8......	17.7	5.77	500	1.013	4.46	3.60	0.360	0.233	0.82	24.0	
Total..................		57.70			41.21					97.0	4.48
Average per day.......		5.77	532	1.013	4.12	3.21	0.440	0.233	0.77		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
57.70	Through the urine...............	41.21
	Through the faeces...............	4.48
	Through the hair (18.6 grams)....	2.15
57.70		47.84

		grams
Nitrogen balance for ten days	=	+ 9.86
Nitrogen balance per day	=	+ 0.99

Intake per day 0.33 gram nitrogen and 78.0 calories per kilo of body-weight.

Bitch 17, period 3

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Totol nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
February 27......	17.5	5.31	840	1.010	3.56	2.54	0.380	0.243	0.91		
February 28......	17.7	5.31	1190	1.008	4.13	3.07	0.440	0.255	0.86		
March 1......	17.7	5.31	910	1.006	3.60	2.84	0.400	0.245	0.70		
March 2......	17.8	5.31	1320	1.006	5.04	3.90	0.550	0.252	1.08		
March 3......	17.8	5.31	1400	1.006	5.22	3.64	0.500	0.253	1.02		
March 4......	17.9	5.31	1050	1.006	3.89	3.02	0.350	0.232	0.94	55.0	
March 5......	18.0	5.31	650	1.010	5.29	4.42	0.370	0.239	0.85		
March 6......	17.9	5.31	1030	1.010	4.70	3.79	0.390	0.250	0.86		
March 7......	18.0	5.31	430	1.016	5.34	4.63	0.350	0.231	0.70		
March 8......	18.1	5.31	610	1.012	5.17	4.58	0.250	0.252	0.71	35.0	
Total		53.10			45.94					90.0	5.94
Average per day		5.31	943	1.009	4.59	3.63	0.398	0.245	0.86		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
53.10	Through the urine................	45.94
	Through the faeces...............	5.94
	Through the hair (29.4 grams)....	3.78
53.10		55.66

Nitrogen balance for ten days = − 2.56 *grams*

Nitrogen balance per day = − 0.26

Intake per day 0.30 gram nitrogen and 72.0 calories per kilo of body-weight.

Bitch 17, period 4

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE: Volume 24 hours	URINE: Sp. gr.	URINE: Total nitrogen	URINE: Urea nitrogen	URINE: Ammonia nitrogen	URINE: Creatinine nitrogen	URINE: Phosphate P_2O_5	FAECES: Weight dried	FAECES: Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
March 27	17.8	5.33	900	1.008	4.92	4.00	0.440	0.277	0.85		
March 28	18.2	5.33	1070	1.007	4.82	3.98	0.410	0.253	0.98	14.0	
March 29	18.1	5.33	1220	1.007	5.46	4.45	0.460	0.268	0.99		
March 30	18.1	5.33	940	1.007	5.28	4.34	0.400	0.245	0.82		
March 31	18.2	5.33	1070	1.010	5.62	4.86	0.470	0.253	0.99	63.0	
April 1	18.1	5.33	770	1.010	6.53	5.46	0.480	0.232	1.00		
April 2	18.2	5.33	1160	1.009	6.86	5.73	0.580	0.241	1.12		
April 3	18.2	5.33	850	1.010	5.89	4.76	0.480	0.232	1.67		
April 4	18.2	5.33	710	1.015	5.85	4.80	0.340	0.240	0.93		
April 5	18.2	5.33	1070	1.007	5.04	4.21	0.470	0.244	1.10	45.0	
Total		53.30			56.27					122.0	8.93
Average per day		5.33	976	1.009	5.63	4.66	0.453	0.248	1.04		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
53.30	Through the urine	56.27
	Through the faeces	8.93
	Through the hair (26.9 grams)	2.77
53.30		67.97

Nitrogen balance for ten days = − 14.67 *grams*

Nitrogen balance per day = − 1.47

Intake per day 0.29 gram nitrogen and 70.0 calories per kilo of body-weight.

Bitch 17, period 5

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
April 24	18.4	5.90	390	1.020	4.92	4.07	0.370	0.216	0.81		
April 25	18.3	5.90	210	1.030	4.35	3.52	0.320	0.216	0.91	39.0	
April 26	18.3	5.90	370	1.018	5.13	4.20	0.420	0.219	0.86		
April 27	18.4	5.90	330	1.023	4.80	3.95	0.370	0.235	0.88		
April 28	18.4	5.90	510	1.020	5.62	4.66	0.340	0.238	0.93	21.0	
April 29	18.4	5.90	210	1.033	4.89	3.92	0.370	0.216	0.77		
April 30	18.4	5.90	200	1.036	5.07	4.15	0.380	0.225	0.96		
May 1	18.4	5.90	200	1.038	5.28	4.48	0.320	0.201	0.76		
May 2	18.4	5.90	220	1.035	5.31	4.46	0.340	0.198	0.78		
May 3	18.4	5.90	210	1.033	5.28	4.41	0.240	0.216	0.85	47.0	
Total		59.00			50.65					107.0	4.91
Average per day		5.90	285	1.029	5.06	4.18	0.347	0.218	0.85		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
59.00	Through the urine	50.65
	Through the faeces	4.91
	Through the hair (27.7 grams)	3.07
59.00		58.63

Nirtogen balance for ten days = + 0.37 *grams*

Nitrogen balance per day = + 0.04

Intake per day 0.32 gram nitrogen and 68.0 calories per kilo of body-weight.

Bitch 17, period 6

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE: Volume 24 hours	URINE: Sp. gr.	URINE: Total nitrogen	URINE: Urea nitrogen	URINE: Ammonia nitrogen	URINE: Creatinine nitrogen	URINE: Phosphate P_2O_5	FAECES: Weight dried	FAECES: Nitrogen
1906	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*grams*	*grams*	*grams*
May 22	18.4	5.90	430	1.020	6.15	4.87	0.530	0.241	0.95	10.0	
May 23	18.4	5.90	300	1.021	5.40	4.19	0.490	0.241	1.13		
May 24	18.4	5.90	380	1.022	5.82	5.07	0.330	0.231	1.12	13.0	
May 25	18.5	5.90	330	1.025	5.97	5.17	0.390	0.219	1.14		
May 26	18.6	5.90	250	1.031	5.31	4.45	0.350	0.225	0.91	25.0	
May 27	18.6	5.90	410	1.020	5.61	4.36	0.410	0.231	1.35		
May 28	18.7	5.90	390	1.020	5.19	4.06	0.350	0.222	0.76		
May 29	18.7	5.90	240	1.030	4.17	3.41	0.280	0.214	0.73		
May 30	18.8	5.90	230	1.027	4.41	3.60	0.270	0.214	0.75		
May 31	18.8	5.90	350	1.020	4.44	3.56	0.250	0.234	0.81	30.0	
Total		59.00			52.47					78.0	5.38
Average per day		5.90	331	1.024	5.25	4.27	0.365	0.227	0.96		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
59.00	Through the urine	52.47
	Through the faeces	5.38
	Through the hair (35.5 grams)	4.33
59.00		62.18

Nitrogen balance for ten days = − 3.18 *grams*

Nitrogen balance per day = − 0.32

Intake per day 0.31 gram nitrogen and 67.0 calories per kilo of body-weight.

Bitch 17, period 7

DATE	BODY-WEIGHT	FOOD NITROGEN	URINE							FAECES	
			Volume 24 hours	Sp. gr.	Total nitrogen	Urea nitrogen	Ammonia nitrogen	Creatinine nitrogen	Phosphate P_2O_5	Weight dried	Nitrogen
1908	*kilos*	*grams*	*cc.*		*grams*	*grams*	*gram*	*gram*	*gram*	*grams*	*grams*
June 17	19.7	5.89	540	1.010	4.00	3.21	0.280	0.235	0.60	21.0	
June 18	19.7	5.89	520	1.010	3.92	3.14	0.170	0.238	0.45		
June 19	19.7	5.89	490	1.010	3.53	2.95	0.360	0.249	0.54	30.0	
June 20	19.8	5.89	450	1.010	3.90	3.04	0.320	0.237	0.53		
June 21	19.9	5.89	350	1.020	3.75	3.00	0.300	0.231	0.63		
June 22	20.1	5.89	700	1.010	4.61	3.90	0.320	0.237	0.76		
June 23	20.1	5.89	500	1.012	4.50	3.35	0.350	0.253	0.53		
June 24	19.9	5.89	450	1.020	4.92	3.99	0.270	0.238	0.68		
June 25	19.9	5.89	320	1.025	4.89	4.00	0.290	0.241	0.66		
June 26	20.0	5.89	480	1.010	4.89	4.12	0.380	0.253	0.71	40.0	
Total		58.90			42.91					91.0	3.98
Average per day		5.89	480	1.014	4.29	3.47	0.304	0.241	0.61		

Nitrogen balance—ten days

Nitrogen taken in	*Nitrogen output*	
grams		*grams*
58.90	Through the urine	42.91
	Through the faeces	3.98
	Through the hair (22.9 grams)	2.86
58.90		49.75

Nitrogen balance for ten days = + 9.15 *grams*
Nitrogen balance per day = + 0.92

Intake per day 0.30 gram nitrogen and 70.0 calories per kilo of body-weight.

Summary—bitch 17

PERIOD MONTH	BODY-WEIGHT	FOOD PER DAY			OUTPUT PER DAY—AVERAGES						N BALANCE + OR −	N UTILIZATION	FAT UTILIZATION
		Total N	N per kilo	Fuel value per kilo	N through kidneys	N through excrement	N through hair	Urea N	Ammonia N	Creatinine N			
	kilos	*grams*	*gram*	*calories*	*grams*	*gram*	*gram*	*per cent*	*per cent*	*per cent*	*gram*	*per cent*	*per cent*
1	18.3	9.06	0.49	80.0	7.73	0.66	0.28				+0.39	92.0	98.0
2	17.6	5.77	0.33	78.0	4.12	0.44	0.21	77.8	10.6	5.6	+1.00	92.0	98.0
3	17.9	5.31	0.30	72.0	4.59	0.59	0.37	78.4	8.8	5.4	−0.24	89.0	94.0
4	18.1	5.33	0.29	70.0	5.63	0.89	0.27	82.8	8.0	4.4	−1.52	83.0	97.0
5	18.4	5.90	0.32	68.0	5.06	0.49	0.30	82.6	6.8	4.3	+0.05	91.0	97.0
6	18.6	5.90	0.31	67.0	5.25	0.53	0.43	81.4	6.9	4.3	−0.31	90.0	96.0
7	19.9	5.89	0.29	70.0	4.29	0.39	0.28	80.8	7.1	5.6	+0.93	91.0	98.0

Summary—bitch 21

PERIOD MONTH	BODY-WEIGHT	FOOD PER DAY			OUTPUT PER DAY—AVERAGES						N BALANCE + OR −	N UTILIZATION	FAT UTILIZATION
		Total N	N per kilo	Fuel value per kilo	N through kidney	N through excrement	N through hair	Urea N	Ammonia N	Creatinine N			
	kilos	*grams*	*gram*	*calories*	*grams*	*gram*	*gram*	*per cent*	*per cent*	*per cent*	*gram*	*per cent*	*per cent*
1	13.8	4.76	0.34	89.0	3.73	0.84	0.15	82.3	9.0	4.0	+0.03	82.0	95.0
2	14.2	4.20	0.30	76.0	4.16	0.29	0.19	86.2	6.5	3.9	−0.45	93.0	98.0
3	14.3	4.80	0.33	77.0	4.80	0.31	0.12	84.5	5.4	3.4	−0.43	93.0	98.0
4	14.2	4.86	0.34	79.0	4.30	0.44	0.25	83.0	7.1	3.5	−0.13	90.0	96.0
5	14.5	4.85	0.33	80.0	3.93	0.86	0.17	83.5	8.0	4.0	−0.11	82.0	93.0
6	15.3	4.85	0.31	76.0	4.25	0.38	0.26	83.9	6.4	3.5	−0.05	92.0	96.0

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THE CONTRACTION OF SMOOTH MUSCLE CELLS IN TISSUE CULTURES

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The smooth muscle cells upon which were made the following observations were obtained from the growth of the amnion of chick embryos in tissue cultures (Lewis and Lewis, 1915).

In a hanging drop of medium the growth adheres closely to the cover slip; the cells are so thin that, if desired, their most minute structure can be observed by means of the oil immersion lens and high oculars. However for general purposes the contracting cell or cells can be clearly seen with the low powers of the microscope.

The amnion of the young chick embryo is composed of a single layer of mesenchyme cells overlaid by a single layer of epithelial cells. The mesenchyme cells differentiate into smooth muscle cells, which undergo definite contraction as early as the fourth or fifth day of incubation. So far as we know there is no nerve supply to the amnion.

In the tissue cultures the smooth muscle cells frequently grow out from the explanted piece unmixed with the epithelial cells. Such a smooth muscle cell can be readily recognized not only by it shape but also by the characteristic refractility of the cytoplasm. The minute structure of these living smooth muscle cells does not differ greatly from that of a living cell in general. The cytoplasm of the smooth muscle cell is more refractile than that of other cells. In addition to the nucleus with its nucleolus, the cytoplasm contains numerous mitochondria, a few neutral red granules and occasionally one or two fat globules. The mitochondria of the smooth muscle cells are more threadlike than are those of the epithelial cells. There is in the living contracting smooth muscle cells no structure which corresponds to the myofibrils of the histological preparation. As far as the changes in shape of the cells during contraction is concerned the nucleus, the nucleolus and the granules of the cell play only a passive part. The marked change

takes place in the cytoplasm itself, which becomes thicker in a certain region where it is usually drawn into folds during contraction and is smoothed out again during relaxation.

The smooth muscle cells of the cultures usually grow out closely attached to the cover slip and, for this reason, each cell becomes flattened out into an exceedingly thin layer. Such a very thin cell occasionally exhibits delicate straight lines or folds of the non-granular cytoplasm as though the cell was tightly stretched in its long axis, i.e., in the direction of migration. Fixation emphasizes these folds and also causes fibrils to appear within the cytoplasm of other smooth muscle cells of the culture, but never within the cytoplasm of the epithelial cells. Although the majority of the smooth muscle cells grow out as large flat cells, there is always a number of thicker, spindle-shaped cells less closely attached to the cover slip; also in practically every culture, there can be found within the new growth, bundles of three or more cells (fig. 3) which have grown out from the explanted piece of the amnion on the under surface of the growth and which are not closely attached to the cover slip but end in the thin flat cells. The smooth muscle cells of the explanted piece contract for some time after explantation and then cease to contract unless stimulated; here and there, however, among the cells of the new growth can always be found a cell or a bundle of cells undergoing rhythmical contraction even after twenty-four to forty-eight hours' growth. Each bundle of cells always contracts for some hours during the growth of the culture. The spindle-shaped cells either contract rhythmically or can be stimulated to such activity. The whole cell is not necessarily involved in this phenomenon and frequently the greater part of the cell remains quiet while one process undergoes rhythmical contraction. The thin, flat cells never contract unless stimulated, and then only when a part of the cell or the whole cell has become less tightly attached to the cover slip.

Rhythmical contraction which involves more or less of the growth and explanted piece, can be inaugurated by greater or less stimulation. After rhythmical contraction has ceased it can be again set up by washing the preparation with a drop of the culture medium to which an increased amount of calcium has been added.

Although the cell can be observed with ease, it is nevertheless difficult to determine just what actually happens during contraction. The microscopical appearance of the contraction can be described as follows: The cytoplasm seems to be drawn together at a given region,

diagrammatically shown in figure 1 as C, so that the cytoplasm becomes thicker and is usually thrown into folds. Beyond this active region there is always a zone where no movement of the cytoplasm occurs, and since in almost every case these quiet zones are fixed, there results an area of tension between the fixed zones (A and B) and C. This area becomes thinner during contraction; in some cases the muscle strand broke here. That is, the muscle substance actually moves away from two regions, A and B, to be piled up in folds at another region, C. The region in which the folds occur, C, has been called the contraction node; it may occur midway between A and B (fig. 1), or more towards one end or the other of the area of activity, as in figure 2.

Coincident with the contraction of the muscle there usually occurs a swaying or pendular movement, so that during contraction the muscle material is drawn together not in a straight line, but more or less in a curve. This pendular movement is more pronounced during relaxation than during contraction.

In some instances the bundle of muscle cells hung below the cover slip in an arc so that during contraction the strand was straightened and stretched more closely to the cover slip while during relaxation it again fell away from the cover slip. In one bundle of cells observed the quiet end, B, beyond the contracting region was free and in this case the whole bundle was drawn back towards the explanted piece during contraction and was stretched out into the medium during relaxation.

During contraction each bundle of cells and each single cell has an individual rhythm and also an individual strength of contraction but the contraction of these cells can be classified in a general way according to the number of cells, or according to the amount of a single cell undergoing contraction. An example of some of the various types of rhythmica contraction exhibited by these smooth muscle cells from the amnion of the chick is given below. The growth in all cases was twenty-four hours old so that in all probability contraction had been going on for many hours before the observations were made.

Case 1. Figure 3 shows a bundle of cells in which the contraction node appeared at one end of the muscle strand.

The movement began in cells within the explanted piece and passed through the three cells each of which moved in turn so that the contraction node occurred at c, where the bundle of cells ended in three processes, each of which was closely attached to the cover slip. The thickening of the muscle strand took place within the three processes and resulted in a peculiar coiling difficult of description, ex-

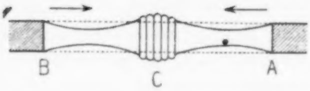

Fig.1

Fig.6.

Fig.2.

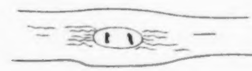

Fig.6a.

Fig.6b.

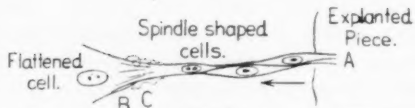

Fig.3.

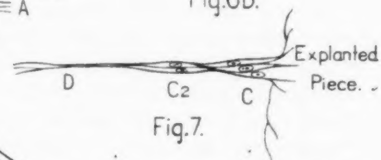

Fig.7.

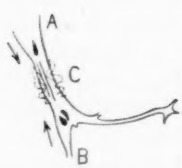

Fig.4.

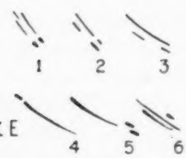

Fig.4a.

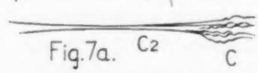

Fig.7a.

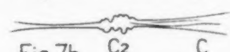

Fig.7b.

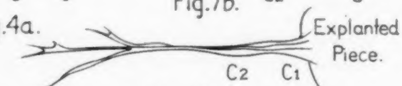

Fig.8.

Fig.5.

Fig.5a.

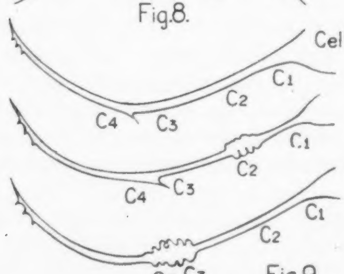

Fig.9.

cept in the rather picturesque language used by one observer, who remarked that it resembled a wriggling mass of worms. Then as relaxation of the bundle took place, the cells became lengthened out and the contracted material was drawn out into three smooth processes. The contraction was rhythmical in that the bundle shortened and thickened with a swaying motion about once every twenty seconds for three hours and then the rate of rhythm slowed to one contraction about every minute. The interval between the contractions could be decreased by warming the preparation. It was difficult to time the rhythm exactly and as nearly as could be observed the shortening lasted one to two seconds, the height of the shortening lasted about one second, while the relaxation required about two to four seconds and was followed by a period of rest. The increase in rate of the rhythm was mostly due to the shortening of the period of rest, although there was also some increase in the rapidity of the actual contraction and relaxation. After some hours not only did the rhythm become slower but there was also a decreased amount of shortening and coincident with this a decreased amount of material thrown into folds in the contraction node. The bundle continued to contract although at the end of twenty-four hours the contractions were rather infrequent, due partly to the enlargement of the growth and to the increase in the number of cells within the bundle and to the close attachment of these cells to the cover slip.

Case 2. Figure 4 shows a process of a cell which was undergoing rhythmical contraction in a twenty-four hour growth from an explanted piece of amnion of a seven day chick embryo. In this process the mitochondria could be clearly seen and their behavior, or rather lack of special behavior, observed during contraction and relaxation.

The drawings were all free hand.

Figs. 1 and 2. A diagram of a contracting smooth muscle cell; *A* and *B* are the regions from which the cytoplasm moves towards *C*; *C* is the region of shortening and thickening which contains the folds or contraction node. The shaded area represents the quiet areas which are usually fixed.

Fig. 3. A bundle of three contracting cells. The contraction node is indicated by the dotted line at *C*. Movement took place from *A* towards *B*.

Fig. 4. A process of a cell in which the movement was from *A* and *B* with the contraction node at *C* midway between *A* and *B*.

Fig. 4 a. Sketches of various shapes in which the mitochondria appear in the process shown in figure 4 during the contraction of the latter.

Figs. 5 and 5 a. Sketches of a migrating cell whose process continued to contract during migration.

Figs. 6, 6 a and 6 b. Nuclear portion of a cell in which a weak contraction was taking place without the appearance of a contraction node.

Figs. 7, 7 a and 7 b. A bundle of five cells in which a contraction node occurred at *C* and was followed by one at C_1 as relaxation occurred at *C*.

Fig. 8. A bundle of cells which swayed back and forth with a pendular motion and exhibited only an occasional shortening and thickening.

Fig. 9. A process of a cell with four different regions at which the contraction node occurred irregularly.

The process was continuous at *B* with another cell, but the wave of contraction did not pass from the one cell to the other. The movement began simultaneously from *A* and *B* and the contraction node occurred at *C* midway between *A* and *B*. At 10 a.m. the contractions took place every eight seconds but later slowed down to one every minute. The mitochondria present in the process at 10 a.m. were in the form of two long threads, one short rod and two large granules, as drawn above. During the contraction of the process the mitochondria were pushed together and the long filament shaped ones became somewhat curled, but they did not undergo any characteristic changes coincident with each contraction except the passive alteration by which the mitochondria appeared slightly shorter during contraction and longer during relaxation. Although the mitochondria remained passive in so far as the contraction was concerned, they were exceedingly active and their shape changed continuously regardless of the contraction of the cytoplasm, so that during a period of about twelve contractions they exhibited the different forms shown in figure 4 a.

The drawings are free hand and indicate only the general change in shape.

The other process shown in the drawing took no part in the contraction.

Case 3. Figure 5 shows a process which contracted rhythmically during the migration of the cell. The process was slowly withdrawn into the cell and the cell changed its shape. In this case there were no mitochondria directly within the contracting area.

The movement took place from *A* towards *B* and from *B* towards *A* and the contraction node occurred at *C* midway between *A* and *B*. The entire cell, with the exception of this one process, remained quiet. The nucleus and mitochondria were clearly and easily observed. No unusual change in the behavior of the nucleus or the mitochondria which differed in any way from that of any cell could be detected and the only means by which the cell could be identified as smooth muscle was by the more refractile cytoplasm and the rhythmically contracting process. The end of the process appeared to be more or less closely attached to the cover slip; the movement began at *B* somewhat proximal to the extremity. The end of the process moved coincidently with the migration of the cell and the whole process was slowly drawn in by the cell during migration so that it became much shorter and broader. The migration and change in shape of the cell was slow; four hours later the cell appeared as in figure 5 a. The process was then broad and flat but the rhythmical contraction still continued between approximately the same points, *A* and *B*. There was a fold present in the surface cytoplasm which became curled during contraction. This fold later disappeared. One hour later the process had become still broader and flatter but the rhythmical contraction continued, although the swelling at *c* was no longer as great nor was the amount of material thrown into folds at *C* as large as it formerly was. The observations were not continued longer. Within the nucleus, the nucleolus or the mitochondria there was no change which could be considered a causal factor of the contraction or relaxation.

Case 4. Figure 6. In this case the rhythmical contraction took place in the region of the nucleus, although there was very little swelling and no appearance of folds. The wall of the cell remained quiet but the cytoplasm in the region of the nucleus flowed together and then flowed back.

The mitochondria were active and continued to change in the manner characteristic of these bodies. At one time three mitochondria united together as shown in figure 6 b and remained so during two contractions. The nucleus remained quiet, but the mitochondria curled on each side of the nucleus coincident with the flowing together of the cytoplasm and then straightened out during relaxation. (Fig. 6 a.)

Observations were made upon cells in other areas where the contraction node occurred across the nucleus but in these cases the behavior of the nucleus and the mitochondria could not be followed.

Case 5. Figure 7 was a bundle of cells observed in a twenty-four hour growth from the amnion of a five day chick embryo. At 10 a.m. the bundle was contracting rhythmically and continued to contract rhythmically for six hours until finally at 4.30 p.m. the strand broke at D and the cells immediately drew back to the explanted piece. Later these same cells developed processes and began to migrate slowly away from the edge of the explanted piece.

At 10 a.m. the bundle was contracting in such a manner that the contraction-node appeared first at C_1; then the bundle relaxed and four seconds later the contraction node appeared at C_2 followed by a relaxation of the bundle. At C_1, where there seemed to be three cells, each cell was thrown into folds, but at C_2 where two nuclei were observed the protoplasm was thrown into only one heap of folds.

Four hours later the contraction node appeared at C_1 two and sometimes three times, at intervals of five seconds before the contraction node appeared at C_2.

Two hours later the interval between the contractions was about the same although the relation had changed. The contraction node appeared at C_1, figure 7 a, and then at C_2 coincident to or just following the relaxation at C_1, figure 7 b, and then while the relaxation took place at C_2 the node appeared again at C_1. This resulted in a contraction node being present either at C_1 or C_2 most of the time. The contraction node did not move along the bundle from C_1 to C_2, but the protoplasm moved together so that the bundle was thrown into folds either at C_1 or C_2. Although these two points did vary slightly, they remained in practically the same general location in the bundles for hours.

Shortly after this the bundle which had become quite thin between d and c broke at D and ceased to contract.

Case 6. Figure 8 was a bundle of cells which closely resembled that of figure 1 and also that of figure 5 except that in this bundle the contraction was mostly of the pendular type. This pendular movement took place without any noticeable shortening or thickening of the bundle. Thus the bundle simply swung back and forth two and sometimes three times without the appearance of a contraction node anywhere along its length. Later contraction nodes appeared at C_1 or at C_2. These were alternated with two or three pendular movements.

Case 7. Figure 9 was a process of a cell the proximal part of which contracted while a distal portion remained quiet. These contractions, which took place every seven seconds, were irregular and were observed carefully for several hours. The protoplasm moved in such a way that the contraction node appeared at C_1, C_2, C_3 or C_4. The movement of the protoplasm was not equal from the two directions but was largely only from the cell-body or A towards the end of the process.

After an hour's observations the explanted piece contracted and a contraction wave passed over the entire cell and process, and the entire process from C_1 to its end was thrown into folds showing that while the distal end of the process did not contract rhythmically it was capable of contraction under a given stimulus. Later the process slowly relaxed and although remaining slightly thicker it behaved as before the contraction of the explanted piece, the points C_1, C_2, C_3 and C_4 being approximately the same.

When the process again began to contract rhythmically the movement of the protoplasm took place every fifteen seconds from the cell body to C_4 and the contraction node was formed at C_4. The process was much thinner during relaxation than during contraction. Fifteen minutes later the contraction again took place irregularly and the contraction node appeared at C_2 or C_3 followed by a relaxation, or else the contraction node appeared at C_2 or C_3 and then at C_2 or C_3 to C_4 without a relaxation. In fact there seemed to be a contraction node present at some point C_1, C_2, C_3 or C_4 all the time without a relaxation and rest of the entire bundle. Sometimes the contraction node occupied all the region between C_3 and C_4.

Dr. Robert Chambers performed a number of microdissection operations upon these cultures in which he found that touching the cells by means of a delicate glass needle resulted in the inauguration of rhythmical contraction in the cell itself or in a process of the cell. The cytoplasm was elastic, that is, a cell or its process when pushed out at an angle at once returned to its former position upon the withdrawal of the needle. Such an operation resulted in rhythmical contraction of the muscle material after it had returned to its former position.

CONCLUSION

The power of rhythmical contraction exists in the smooth muscle cell of the cultures of the chick amnion as an inherent property of the protoplasm of the smooth muscle cell and may be exhibited either by a bundle of cells, by an individual cell or by a part of a single cell. When this phenomenon has ceased it can again be instituted either by touching the cell or by the addition of calcium to the culture medium

The cytoplasm itself plays the most active part in the contraction, while so far as can be seen, the nucleus, the nucleolus and the mitochondria take only a passive part in the contraction. There are no myofibrils present in these living contracting smooth muscle cells. The cytoplasm is elastic. There is a pendular movement of the muscle strand coincident with the shortening and thickening of the cytoplasm. The cytoplasm is drawn into folds to form the contraction node.

THE EFFECT OF THE EMOTIONS ON THE CATALASE CONTENT OF THE LIVER

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Several observers have shown that the emotional states affect the internal as well as the external secretions. Of the older observers the work of Spallanzani, Beaumont, Bidder and Schmidt, and of Richet (1) may be mentioned. It is to Pawlow (2), however, that our more exact knowledge concerning the effect of the emotions on the secretions is due. Pawlow showed that pleasurable emotions such as are aroused by the sight, smell and taste of appetizing food produced a flow of the digestive juices while such emotions as pain, anger and fear inhibited the flow. He showed also that this so-called "psychic secretion" was produced by reflex stimulation through the autonomic nervous system. Dreyer (3) demonstrated that artificial stimulation of the branches of the splanchnic nerves supplying the adrenals increased the output of adrenalin into the blood. Oliver and Schäfer (4) showed that injection of adrenalin brought about a vasoconstriction in the internal vegetative organs such as the spleen, kidney and intestines, thus driving more blood into the skeletal muscles, heart and central nervous system. Cannon and de la Paz (5) demonstrated that the secretion of the adrenal bodies was increased during emotional excitement and that this was due to a reflex stimulation of these glands through the splanchnic nerves. In his book, *Bodily Changes in Pain, Hunger, Fear and Rage,* Cannon cites many instances of the adaptative relation of the increased output of adrenalin during emotional excitement. The increased secretion functions in the manner pointed out by Oliver and Schäfer, namely, by driving the blood from the abdominal viscera into the organs called upon in emergencies, such as the central nervous system, the lungs, heart and active skeletal muscles; it thus renders the organism more effective in the physical struggle. Crile has also pointed out similar bodily changes in his *Origin and Nature of the Emotions* and in his *Mechanistic View of War and Peace.*

We showed that when oxidation is increased, as, for example, by increasing the amount of work, by thyroid feeding, etc, the catalase content of the muscles is correspondingly increased, and that when oxidation is decreased, as, for example, by decreasing the amount of work, the catalase content is decreased (6). From this and similar evidence the conclusion was drawn that the catalase content of a tissue is an index to the amount of oxidation in the tissue, being greatest in amount where oxidation is greatest and least where oxidation is least. It may be that oxidation in the tissues is made possible by catalase in the manner suggested by Bach and Chodat (7), namely, that the catalase acts on an organic peroxide, comparable in structure to hydrogen peroxide, and liberates atomic oxygen for the oxidative processes. The object of the present investigation was to determine the effect of stress and combat, where extreme muscular exertion is put forth by the animal, on the catalase content of the different organs and tissues of the body with the hope of determining if catalase is formed in situ in the tissues or if it is formed by a particular organ and given off to the blood. At the beginning of this work the catalase content of several organs and tissues was determined but it was soon found that the emotions affected very quickly and extensively the catalase of the liver so our attention was directed particularly to the effect on this organ.

The animals used were cats. We were fortunate in being able to obtain from one home ten young cats, each about three months old, that had lived under practically the same conditions. Fortunately also there was in our possession a dog with a particularly strong dislike for cats. Five of the young cats were placed in a small cage made of strong, fine-meshed wire and the dog was turned loose and permitted to bark and bite at the cats in the cage for one hour a day for two consecutive days. All the young cats showed fight and great excitement under these conditions. At the end of the frightening process on the second day the cats were etherized and the blood vessels of the livers washed with large quantities of 0.9 per cent sodium chloride until they were free of blood, as was indicated by the fact that the wash water gave no test for catalase. The livers were then removed and ground up separately, first in a hashing machine, then in a mortar and finally pressed through one thickness of a linen towel of medium weight. The catalase content of the livers was determined by adding 1 gram of the prepared material to 700 cc. of hydrogen peroxide at 22°C. in a large bottle, and as the oxygen gas was liberated it was conducted

through a rubber tube to a large inverted graduated cylinder previously filled with water. The amount of oxygen gas liberated by a gram of the material in ten minutes was read off directly from the graduated cylinder where it had displaced the water. After this volume was reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the gram of material. A full description of the method may be found in a previous publication. The results of these determinations are given in table 1 after "fighting cat." Similarly the catalase content of the livers of the five young cats that had not undergone the frightening process was determined. The results of these determinations are given in the table after "normal cat." It may be seen that the average amount of oxygen liberated by the livers of the normal cats was 1588 cc., while that liberated by the fighting cats was 3036 cc. By comparing these amounts of oxygen it will be seen that the catalase content of the livers of the fighting cats had been increased by about 90 per cent over that of the normal cats.

TABLE 1

After normal and fighting cat are given the number of cubic centimeters of oxygen liberated from 700 cc. of hydrogen peroxide by gram of the livers of these animals in ten minutes

	CAT					AVERAGE AMOUNT OF OXYGEN IN CUBIC CENTIMETERS
	1	2	3	4	5	
Liver						
Normal cat	1400	1476	1650	1875	1540	1588
Fighting cat	2875	3390	2800	3175	2940	3036

That the catalase is formed in the liver and is given off to the blood is made still more probable by the following facts: The catalase of the liver was found to be much more resistant to oxidation than that of the blood or tissues. A description of the method of destroying catalase by oxidation is given in a previous publication (8). It was noticed that the catalase of the blood of the fighting cats took on the character of that of the liver in that it was more difficult of destruction by oxidation than the blood catalase of the normal cats. The more difficult destruction of the liver catalase by oxidation is explained on the assumption that it was freshly formed while the catalase of the

blood having been poured out by the liver was older and already in a state of deterioration. As to the mechanism by which the fighting emotions caused an increase in catalase in the liver, it is quite likely that it is similar to the mechanism for the other secretions, namely, reflexly, through the autonomic nervous system. However, further work is necessary to establish this assumption. The value of the increased catalase in the liver and of its discharge into the blood in time of stress and combat would seem to be to facilitate oxidation in the muscles to which the catalase is transported by the blood, thus making available more energy for the fight.

Of the great number of adult cats used in the preliminary work of this investigation it was found that the catalase of the livers of some was increased tremendously by the frightening process while that of the livers of others was not increased so much. We soon observed that of the adult cats some became very much frightened and fought at the dog very violently, while others fought less violently and that some of the cats paid very little attention to the dog. We assumed that the lack of uniform increase of catalase in the livers of the adult cats that had undergone the frightening process was due to the different degrees of excitement produced in the cats. As a matter of fact, in order to obtain uniform results for the normal cats it was necessary to avoid exciting them unduly when catching and conveying them to the laboratory.

In addition to the work on cats, we tried out the effect of exercise, which consisted in running, on the catalase content of the livers of dogs. The dogs were made to run at a fairly good speed by being led by a person in an automobile. It was found that running for a distance of ten miles on two consecutive days increased the catalase of the livers of dogs by about 40 per cent. Since only four dogs were used in these experiments, two for control and two for exercise, further work on this problem would seem desirable.

CONCLUSIONS

Evidence is presented in this paper to show that the fighting emotions, and probably exercise, increase greatly the catalase content of the liver and that this catalase is given off to the blood and is carried to the tissues to be used presumably in producing increased oxidation.

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CONCERNING THE ELECTRODES USED IN ELECTROCARDIOGRAPHY

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In electrocardiographic work it is necessary to use a non-polarizable electrode as a connection between the patient and the wires leading to the galvanometer, in order to avoid the distortion of the electrocardiogram which polarization at the electrodes might produce. This electrode has usually taken the form of some modification of the Du Bois-Reymond principle. As used by Einthoven and others, it consists of two receptacles, one being a porous cup within the other. One contains a strong solution of sodium chloride, in which a part of the limb is immersed; the other a solution of zinc sulphate and an amalgamated zinc plate, from which the current is led off. It is a matter of indifference which vessel contains which solution.

An electrode was devised by H. B. Williams (1) which had the great advantage of dispensing with jars for the solutions and thus making the application of the electrode much more simple and the apparatus much less cumbersome. It was stated that the electrode was quite satisfactory for electrocardiographic work, but the control curves were not published to show this. The original form of this elect ode has been modified by Doctor Williams so that it now consists of a p ate of German silver about 12 by 25 cm. and thin enough to be read l l bent around its long axis so as to be fitted over the limb. The wii ys are attached to a stem of the same metal which should be rivetere, not soldered, to the plate. If solder is used it becomes wet with thed solution and forms an electric couple which may produce a very large current to be compensated. To apply the electrode, the limb is covered from wrist to elbow or from ankle to knee by a bandage wet with a strong solution of sodium chloride, the metal plate is placed over this and the bandage is continued over its outer surface. It has been found necessary, as pointed out elsewhere (2), to have the salt solution as warm as possible though this is to facilitate the passage of the current

through the skin of the patient and is wholly unnecessary as far as the electrodes are concerned.

Lewis has lately published a note (3) emphasizing the importance of using non-polarizable electrodes for electrocardiographic work, basing this upon a comparison of non-polarizable with platinum electrodes. Lest this should lead to confusion and result in the universal adoption for clinical work of the cumbersome non-polarizable type of electrode, it seems worth while to publish some tests of the German silver electrode which show its fitness.

If two of the German silver plates are covered by wet bandages and the two bandages are placed in contact so that a strip 2 or 3 feet long

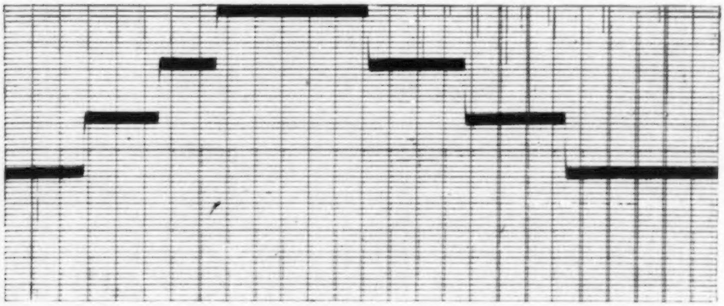

Fig. 1. Application of successive millivolts in series with galvanometer and two German silver electrodes connected by bandages. Record to be read from left to right. Resistance through electrodes and bandage was 1500 ohms. Time interval between the corresponding line of successive pairs of ordinates was 0.20 second. Distance between abscissae—1 mm. in the originals—represents one millivolt.

connects them, the resistance, as measured by substitution, will be about 1000 ohms, the exact amount depending on the wetness of the bandage and its width. In using the method of substitution the electrodes are placed in series with a galvanometer, a standard current is introduced and the deflection of the galvanometer is noted. The electrodes are then removed, a resistance box is placed in the circuit, the standard current is again introduced, and the resistance is varied until the former deflection of the galvanometer is obtained. The amount of resistance thus found represents the resistance of the electrodes. Figure 1 shows a series of steps due to the successive applica-

tion and removal of three units of one millivolt each in series with the electrode couple and galvanometer. These steps are quite like those obtained when two non-polarizable cells are used in the same way, as can be seen by reference to a curve published by Lewis which it is unnecessary to reproduce. It can be seen on close inspection that there is a very slight return of the line of the record from the position to which it has jumped toward its former level. This amounts to about 0.1 mm. on the first step, 0.2 mm. on the second and 0.3 mm. on the third, but it will be seen that the movement is very gradual. The return is due to the polarization of the electrode and it can be readily

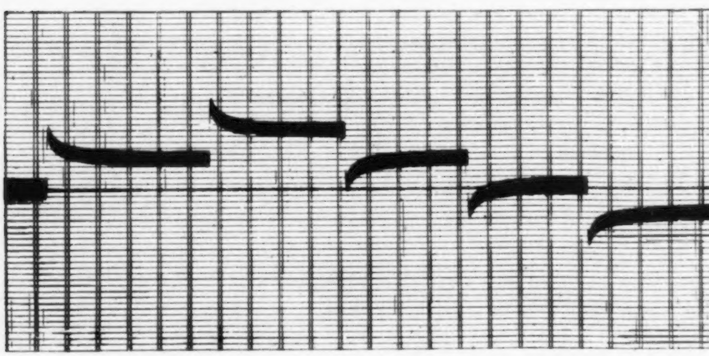

Fig. 2. Application of successive millivolts as in figure 1 except that area of contact on German silver plates was reduced to 8 sq. mm. After this record was taken the string was at such a tension as to jump 10 mm. per millivolt through a resistance of 1800 ohms.

appreciated that it is so slight and so slow to appear as to be of no importance in the course of the QRS complex, and even in the slower P, T and U waves.

The relative freedom of these electrodes from polarization is believed to be due to the smallness of the electric currents concerned and to the large area of the metal in contact with the salt solution. A polarization effect can be produced by connecting two of the electrodes by a bandage which has only a small area of contact with each electrode. Figure 2 shows the result of such an experiment where the total area of contact was only 8 sq. cm. The resistance is not greatly increased, as can be seen by the magnitude of the initial jump, but it can be read-

ily appreciated that such a degree of polarization as is shown by the returning curve after each jump would greatly distort the electrocardiogram.

It would appear from the work of Cowl (4) that the degree of polarization of zinc electrodes immersed in salt solution is very much less than that of German silver electrodes, being almost nil under the conditions of his experiments. This point has been investigated by the author by a method similar to that used to obtain figure 1, and it was found that polarization of the zinc electrodes did not appear when the area of contact with the salt solution was reduced to 50 sq. mm., while with German silver electrodes it was very marked with an area of contact of 190 sq. mm. This would suggest that zinc electrodes should be used for special leads when the area of contact with the metal is to be small.

When platinum electrodes were used, Lewis's curves showed a deflection of 10 mm. declining to 5 mm. in 0.30 second, and one of 5 mm. declining practically to zero in 0.06 second. He says nothing about the size of his electrodes, but since platinum electrodes are usually not of the dimensions of our German silver plates, his results may safely be ascribed to the small area of the contacts. The distortion of the electrocardiogram would be well marked with such a grade of polarization, as pointed out by Lewis, but the German silver plates used as here described do not polarize to a degree which need be considered at all in electrocardiographic work.

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THE RESPONSE OF THE RESPIRATORY MECHANISM TO RAPID CHANGES IN THE REACTION OF THE BLOOD

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For the last year and a half studies have been made of the reaction of the blood by two parallel methods: the Fridericia (1) method for alveolar CO_2 and the Van Slyke (2) method for the determination of the carbon dioxide combining power of the blood. It is probable that this combination offers a simple method for the determination of the state of the respiratory mechanism and its response to variations in the reaction of the blood.

The methods employed in obtaining and testing the specimens were the same as those described in a previous work (3). Only those results were accepted in which it was reasonably sure that both the alveolar and plasma determinations were correct. No alveolar figures have been accepted unless the analyses of at least two specimens agreed within 0.2 to 0.3 volume per cent. In almost every case the blood was withdrawn under a layer of albolene without coming into contact with air and was centrifugated in the tube into which it was drawn.[1] (This was done to prevent the escape of CO_2 from the blood and the consequent shifting of carbonates from the plasma into the cells.) In the few cases in which albolene was not employed the blood was centrifugated within fifteen minutes. This short period has been found insufficient to permit a shifting of the carbonates when the surface of the blood exposed to the air is small, as it is in a centrifuge tube.

According to theory the alveolar carbon dioxide tension should vary directly with the carbonate content of the blood, if the respiratory mechanism and the sensibility of the respiratory center to the H-ion concentration of the blood are normal. Conversely, if the two methods

[1] For this purpose a modification of the "pifflicator" suggested by Dr. Van Slyke was used.

do not agree, some disturbance of the respiratory center may be assumed to exist. In no normal person under natural conditions, at rest, has there been found in these observations a disagreement of over 10 per cent between the alveolar and plasma values. That is, the ratio of alveolar CO_2: plasma CO_2 lies between 0.90 and 1.10. Table 1 demonstrates this clearly. Though the number of cases and determinations is not very great, it is sufficient, nevertheless, to allow one to draw conclusions because there is not a single exception and it is improbable that any combination of circumstances could occur by which such a number of exact agreements could be obtained if the relation were not

TABLE 1

CASE NO.	DATE	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO
1	June 10	45.5	46.2	0.99
2	August 31	47.2	47.6	0.99
	September 4	44.1	43.1	1.02
3	August 15	45.8	42.0	1.09
	August 31	47.6	45.0	1.06
	September 4	46.0	41.7	1.10
	March 20	44.8	42.4	1.06
	June 2	44.4	45.5	0.98
	November 7	42.7	43.4	0.98
	November 8	44.3	43.9	1.01
	January 10	44.1	44.5	0.99
	January 19	44.0	41.3	1.06
	January 29	45.9	45.9	1.00
	February 7	45.3	46.9	0.97
4	November 16	46.3	45.2	1.03
	November 16	43.0	42.5	1.01
	November 17	40.2	42.7	0.94
	November 17	48.5	45.2	1.07
5	March 14	45.3	46.9	0.97
6	November 7	49.7	49.0	1.01
7	March 20	48.7	46.9	1.04
8	March 20	50.2	49.4	1.01
9	January 16	50.2	51.1	0.98
	January 17	43.9	48.7	0.90
10	January 16	46.1	47.6	0.97
	January 20	46.4	45.9	1.01
11	February 2	44.9	46.2	0.97
12	April 15	42.4	42.7	0.99

a physiological one. Examination of table 2 also makes it evident that the presence of the normal ratio is not an individual peculiarity. The ratio has been disturbed in normal people by certain definite methods of procedure that will be considered later.

In other words the theory of the physiological interdependence of the plasma carbonates and the alveolar carbon dioxide is established, the constant which Van Slyke uses to translate the carbonate concentration to terms of alveolar CO_2 tension is correct and the limit of

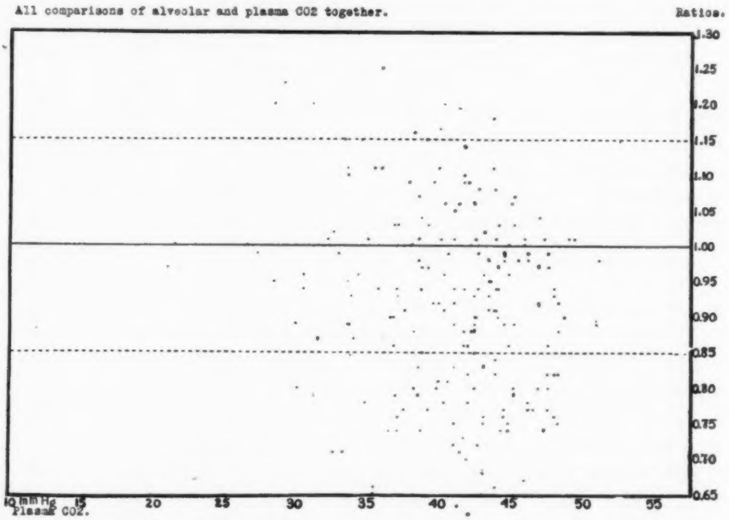

Fig. 1. The abscissa represents plasma CO_2, the ordinate the ratio of alveolar CO_2 to plasma CO_2.

variation in normals under natural conditions at rest is 10 per cent. However, to allow sufficient latitude 0.85 and 1.15 have been taken as the extreme limits of normal. This has been done not only because it is wise to leave a margin of safety but also because, when all the observations have been arranged in the order of magnitude of their ratios as has been done in chart 1, there seems to be a distinct scattering above and below these points. Further study of this chart reveals the fact that both the agreement and disagreement are of the same order of magnitude at all levels of blood reaction.

Table 2 is a tabulation of all the results obtained that could be accepted. In all there were two hundred and twenty-seven comparisons in one hundred and forty of which, or 62 per cent, normal ratios were observed. This low percentage is due to the fact that an attempt was made to study certain types of cases and certain conditions in which a disturbed ratio was to be expected and no effort was made to improve the normal average. Seventy-three cases were studied, of which twenty were cardiacs or had very severe pulmonary lesions. The cardiac cases have been discussed at length elsewhere (3) and will be only briefly mentioned here. Of the remaining fifty-three there were twelve normal persons, twenty-one diabetics, seventeen nephritics, one case of arthritis deformans, one case of gout and one case of starvation acidosis. One hundred and sixty-nine of the determinations were made on these fifty-three cases. One hundred and twenty-one or 74 per cent of these presented no discrepancies. In only three persons was there a constant deviation from a normal relation. In two of these only single determinations were made and in the other the study was continued for only a short period.

The discrepancies observed seem to fall into two large classes: (1) Those due to mechanical factors interfering with the gaseous exchange; and (2) Those due to changes in the control of the respiratory mechanism.

The first class is best illustrated by patients with cardiac dyspnea or very great diminution of the pulmonary capacity. In these the alveolar CO_2 was invariably lower than that of the plasma. If compensation was established the two again came into agreement. This we have shown (3) is due to an impairment of the lungs that renders the excretion of CO_2 more difficult. In response to stimulation of the respiratory center by the retained CO_2, the body overcomes this impairment by increasing the pulmonary ventilation. In this way a pressure difference between the carbon dioxide tension in the blood and that in the alveoli, sufficient to compensate for the impairment in the lungs, is established. The increased minute volume, the intolerance to carbon dioxide in the inspired air (4), the dyspnea and the disproportionate respiratory response to exercise are all expressions of the same thing.

It was impossible to offer the same explanation for the other cases because no similar anatomical disturbance could be discovered. In fact, by the proper procedure discrepancies could be developed in normals. Moreover, the ratios lay above 1.15 as well as below 0.85.

TABLE 2

CASE NO.	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO
Normal persons			
1	45.5	46.2	0.99
2	47.2	47.6	0.99
	44.1	43.1	1.02
3	42.4	42.7	0.99
4	45.8	42.0	1.09
	47.6	45.0	1.06
	46.0	41.7	1.10
	44.8	42.4	1.06
	44.4	45.5	0.98
	42.7	43.4	0.98
	44.3	43.9	1.01
	39.2	35.4	1.11
	44.3	39.9	1.11
	42.9	40.3	1.06
	46.1	42.7	1.08
	38.8	38.5	1.01
	44.1	44.5	0.99
	44.0	41.3	1.06
	51.9	43.9	1.18
	45.8	38.7	1.18
	45.9	45.9	1.00
	50.7	54.3	0.93
	49.1	55.0	0.90
	44.4	48.3	0.92
	45.3	46.9	0.97
5	46.3	45.2	1.03
	40.2	42.7	0.94
	48.5	45.2	1.07
	43.0	42.5	1.01
	39.5	44.5	0.89
	38.2	33.3	1.15
6	45.3	46.9	0.97
	45.0	44.8	1.00
7	49.7	49.0	1.01
8	48.7	46.9	1.04
	48.0	43.4	1.11
	43.8	39.6	1.09
9	50.2	49.4	1.01
	47.9	47.3	1.01
	43.2	41.0	1.05
10	50.2	51.1	0.98
	43.9	48.7	0.90

CASE NO.	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO
Normal persons—continued			
11	46.1	47.6	0.97
	46.4	45.9	1.01
12	44.9	46.2	0.97
Diabetics			
13	43.3	46.9	0.92
	45.3	44.1	1.03
14	44.8	48.0	0.93
15	41.3	37.8	1.09
16	37.1	42.2	0.88
17	47.5	41.7	1.14
18	37.6	38.5	0.98
19	37.0	33.6	1.10
	43.0	44.1	0.97
20	38.1	37.1	1.03
21	28.7	30.5	0.94
	26.6	26.6	1.00
	15.1	19.6	0.76
	23.5	35.4	0.66
	22.9	35.4	0.65
	30.6	49.8	0.61
	33.8	52.5	0.64
	33.0	52.6	0.63
	36.1	46.2	0.78
	37.7	41.0	0.92
	36.8	40.3	0.91
	33.7	42.4	0.80
22	31.8	39.8	0.80
	28.8	43.9	0.66
	33.5	44.8	0.75
23	34.1	28.4	1.20
24	47.5	41.7	1.14
	44.9	35.9	1.25
	41.9	35.9	1.11
	44.9	39.0	1.15
	44.3	38.2	1.16
25	37.5	42.4	0.88
	45.0	42.4	1.06

TABLE 2—Continued

CASE NO.	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO
Diabetics—continued			
25	45.5	41.7	1.09
	37.6	42.4	0.89
	40.0	43.8	0.91
	36.7	43.9	0.84
	39.2	43.9	0.89
	42.4	43.4	0.98
26	40.2	38.7	1.04
27	35.9	41.7	0.86
	37.8	36.8	1.03
	18.2	20.7	0.88
	21.4	31.2	0.69
	36.4	39.6	0.92
	34.9	47.3	0.74
28	40.7	36.8	0.90
	34.1	44.5	0.77
	39.4	48.3	0.82
	36.2	48.3	0.75
	42.6	43.8	0.97
	43.9	44.5	0.99
29	40.6	40.1	1.01
	39.3	42.4	0.93
	35.7	41.7	0.86
	42.8	44.8	0.96
30	21.1	20.3	1.04
	20.4	21.0	0.97
	23.8	30.1	0.80
	27.1	28.4	0.95
	27.1	31.5	0.87
	27.1	34.8	0.78
	23.2	32.6	0.71
	31.6	40.3	0.78
	32.7	40.6	0.81
	38.4	44.8	0.86
	34.0	41.0	0.83
	35.9	44.1	0.81
	36.4	41.0	0.89
	35.6	43.1	0.83
	35.6	45.2	0.79
	35.9	43.9	0.82
	29.5	41.0	0.72
Diabetics—continued			
30	30.2	39.2	0.77
	34.5	42.0	0.82
	35.8	43.1	0.83
	30.6	45.9	0.67
31	33.7	35.7	0.94
	33.0	38.8	0.85
	32.7	34.3	0.96
	32.0	38.5	0.83
	41.2	43.9	0.94
	40.1	45.2	0.89
	38.1	39.2	0.97
	38.0	42.4	0.90
	43.9	44.5	0.99
32	40.1	40.6	0.99
	33.1	44.8	0.74
	41.1	43.4	0.95
33	40.5	39.2	1.03
Nephritics			
34	38.3	42.5	0.90
35	41.2	43.1	0.96
	47.1	43.8	1.08
36	36.3	38.7	0.94
37	33.1	32.6	1.02
38	29.5	34.0	0.87
39	38.4	41.0	0.94
40	39.3	41.7	0.94
41	37.2	42.4	0.88
	40.5	43.4	0.93
42	41.9	41.0	1.01
43	39.7	44.1	0.90
44	27.1	27.3	0.99
45	21.6	21.4	1.00
46	34.5	37.8	0.91
47	33.6	36.6	0.90
	39.7	34.5	1.15
	36.5	35.0	1.01
	41.0	38.5	1.07
48	32.6	33.6	0.95

TABLE 2—Continued

CASE NO.	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO
	Nephritics—continued		
48	30.0	33.6	0.89
	31.5	36.1	0.87
	30.8	42.7	0.72
49	32.4	32.2	1.01
	30.0	33.7	0.89
	45.5	50.9	0.89
	37.2	31.1	1.20
50	35.4	46.2	0.77
	27.5	31.5	0.87
	Arthritis deformans		
51	41.6	43.6	0.95
	Starvation acidosis		
52	35.7	29.1	1.23
	29.4	30.5	0.96
	37.4	33.6	1.11
	Gout		
53	35.2	47.3	0.74
	Cardiacs		
54	37.4	46.9	0.80
	36.2	42.0	0.86
	32.7	44.3	0.74
	32.8	42.0	0.78
55	33.5	38.2	0.88
	32.3	43.1	0.75
	30.8	41.0	0.75
56	28.6	38.7	0.74
	31.3	33.6	0.93
	32.8	32.9	0.99
57	27.5	37.1	0.74
	28.9	37.5	0.77
	34.6	37.1	0.93
	38.0	37.8	1.00
	37.7	38.7	0.97
58	27.1	36.4	0.74
59	39.5	48.0	0.82
	38.9	47.6	0.82
	Cardiacs—continued		
60	32.9	46.6	0.77
	38.5	40.3	0.96
	29.5	41.7	0.71
61	40.9	47.6	0.86
	45.2	48.0	0.94
	38.3	47.6	0.80
	43.1	46.9	0.92
	41.5	44.1	0.94
62	30.4	38.5	0.79
	36.8	39.9	0.92
	30.4	38.2	0.80
	30.7	41.7	0.73
	36.9	48.0	0.76
	42.3	48.3	0.88
	43.7	44.5	0.98
63	39.9	43.9	0.91
64	34.6	46.2	0.75
	36.3	45.2	0.80
65	29.4	43.1	0.68
66	35.4	45.2	0.79
	31.3	46.2	0.68
	36.2	42.4	0.85
67	31.4	36.8	0.86
	34.9	37.0	0.94
68	28.1	37.1	0.76
	32.6	46.9	0.70
69	23.9	33.3	0.71
	30.4	38.5	0.79
	37.2	49.4	0.75
	39.7	43.4	0.91
	32.9	43.1	0.76
70	27.0	38.2	0.71
	Severe pulmonary cases		
71	33.7	44.5	0.76
	32.3	39.9	0.81
	29.2	36.9	0.79
72	25.8	41.3	0.63
73	27.8	49.0	0.57
	33.4	48.3	0.69
	36.6	47.6	0.77
	40.2	49.2	0.82

It seemed more reasonable to suppose that they were due to changes in the mechanism that controls the respirations or to some functional insufficiency of the whole respiratory system or to both factors.

In table 3 are placed all those cases other than patients suffering from cardiac or pulmonary disease that presented discrepancies at any time. Altogether there were forty-eight abnormal ratios in eighteen cases. Careful study revealed the fact that all but three of these followed distinct and considerable changes in the plasma carbonates.

Very early in this study it was found that, after the administration of bicarbonate for therapeutic purposes the plasma carbonates increased more rapidly than the alveolar CO_2. At first this was taken to be a specific carbonate effect, but later the same condition was found to occur after spontaneous recovery from acidosis. This directed attention to the study of the changes in the plasma carbonates and the alveolar CO_2 after all alterations of blood reaction. It developed that the respiratory response was almost never immediate nor complete, but that the alveolar carbon dioxide seemed to lag behind.

The conditions chosen for study were: exercise, the reaction to adrenalin, the spontaneous recovery from diabetic acidosis and the effects of bicarbonate on diabetics, nephritics and normal individuals. It has been difficult to find material for the study of the development of severe and prolonged acidosis, but some suggestive results have been obtained.

The first two cases in table 3 present the effects of exercise in two normal persons. The changes are shown graphically in figures 2 and 3. There was a sharp initial rise in the alveolar CO_2 during the exercise succeeded very rapidly by a fall to a point below the original value. On the other hand the plasma showed a slight drop from the beginning. The slight initial rise in the plasma of case 4 probably does not represent a real increase of blood alkalinity, but is only an expression of the Zuntz (5) reaction (a shifting of the carbonates from the cells to the plasma in response to the increased carbon dioxide). To demonstrate this it was only necessary to aerate the whole blood instead of the plasma with alveolar CO_2, as was done in case 5, figure 3. This initial rise then disappeared.[2]

[2] This method of demonstrating supersaturation with CO_2 is useful only when comparisons of blood from the same individual are made at short intervals. The differences discovered are not sufficiently striking to allow conclusions to be drawn on isolated specimens of blood.

TABLE 3

All cases except cardiacs that show discrepancies

CASE NO.	DIAGNOSIS	DATE	TIME	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO	PROCEDURE AND REMARKS
			The effects of exercise				
4	Normal	January 19	3.00 p.m.	44.0	41.3	1.06	
			3.15 p.m.	51.9	43.9	1.18	Immediately after running up four flights of stairs. Dyspnea quite severe
			3.20 p.m.	45.8	38.7	1.18	When subjective dyspnea had disappeared
6	Normal	March 14	3.00 p.m.	45.3	46.9	0.97	
			3.10 p.m.	55.0+			Immediately after running up three flights of stairs. Dyspnea very considerable
			3.11 p.m.		44.1		Dyspnea very considerable
			3.12 p.m.	42.8			Dyspnea very considerable
			3.15 p.m.	43.5			No subjective dyspnea
			3.17 p.m.	45.0			No subjective dyspnea
			3.18 p.m.		44.8	1.00	
			3.19 p.m.	45.0			
			The effects of adrenalin				
4	Normal	November 8	9.00 a.m.	44.3	43.9	1.01	
			10.00 a.m.	39.2	35.4	1.11	Adrenalin Mxx intramuscularly at 9.30 Very marked hyperpnea
			11.00 a.m.	44.3	39.9	1.11	All symptoms gone
			1.00 p.m.	42.9	40.3	1.06	
			4.00 p.m.	46.1	42.7	1.08	
		November 9	9.00 a.m.	38.8	38.5	1.01	After fasting 36 hours

24	Diabetes	November 2	8.30 a.m.	47.5	41.7	1.14	After fasting 36 hours
			10.30 a.m.	44.9	35.9	1.25	Adrenaline Mxx intramuscularly at 9 a.m. Hyperpnea quite evident
			12.30 p.m.	41.9	35.9	1.11	All symptoms gone
			4.30 p.m.	44.9	39.0	1.15	
		November 3	9.30 a.m.	44.3	38.2	1.16	After fasting 60 hours
			The effects of starvation in normal persons				
5	Normal	November 16	9.00 a.m.	46.3	45.2	1.03	After 14 hours fast
		November 17	9.00 a.m.	39.5	44.5	0.89	After 38 hours fast
		November 18	9.00 a.m.	38.2	33.3	1.15	After 62 hours fast
8	Normal	March 20	4.00 p.m.	48.7	46.9	1.04	
		March 21	4.00 p.m.	48.0	43.4	1.11	After 21 hours fast
		March 23	5.30 p.m.	43.8	39.6	1.09	After 71 hours fast
			Two cases with high ratios and acidemia				
23	Diabetes	October 7		34.1	28.4	1.20	On admission to the hospital
52	Starvation acidosis	December 29		35.7	29.1	1.23	On admission to the hospital. Vomiting continuously
	Vomiting	December 31		29.4	30.5	0.96	Vomiting subsiding
			Cases that received sodium bicarbonate				
4	Normal	January 29	2.00 p.m.	45.9	45.9	1.00	
							$NaHCO_3$ gram 10 by mouth at 3 p.m.
			4.00 p.m.	50.7	54.3	0.93	
							$NaHCO_3$ gram 10 by mouth at 5 p.m.
			6.00 p.m.	49.1	55.0	0.90	
		January 30	10.30 a.m.	44.4	48.3	0.92	Seventeen and one-half hours after last dose of bicarbonate

TABLE 3—continued

CASE NO.	DIAGNOSIS	DATE	TIME	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO	PROCEDURE AND REMARKS
Cases that received sodium bicarbonate (continued)							
47	Chronic nephritis	October 2		33.6	36.6	0.90	
		October 7		39.7	34.5	1.15	After 20 grams of $NaHCO_3$ by mouth
48	Chronic nephritis	June 2		32.2	33.6	0.95	
		June 7		30.0	33.6	0.89	$NaHCO_3$ gram 10 every day, begun
		June 14		31.5	36.1	0.87	
		June 22		30.8	42.7	0.72	Last day of administration of bicarbonate
50	Chronic nephritis	April 5		35.4	46.2	0.77	After recovery from acidemia under bicarbonate treatment
		April 16		27.5	31.5	0.87	Recurrence of acidemia
49	Chronic nephritis	September 3		32.4	32.2	1.01	
		September 10		30.0	33.7	0.89	
		November 7		45.5	50.9	0.89	After administration of bicarbonate
		November 24		37.2	31.1	1.20	One day after resumption of bicarbonate succeeding a period without alkali
21	Diabetes	December 8		28.7	30.5	0.94	Received 755 grams $NaHCO_3$ from December 8 to December 20, inclusive
		December 10		26.6	26.6	1.00	
		December 16		15.1	19.6	0.76	
		December 17		23.5	35.4	0.66	
		December 18		22.9	35.4	0.65	
		December 19		30.6	49.8	0.61	
		December 20		33.8	52.5	0.64	
		December 22		33.0	52.6	0.63	

21	Diabetes	December 23		36.1	46.2	0.78	
		December 24		37.7	41.0	0.92	
		December 26		36.8	40.3	0.91	
		December 30		33.7	42.4	0.80	
22	Diabetes	October 28		31.8	39.8	0.80	Received large doses of $NaHCO_3$ just before admission to the hospital on October 28
		October 29		28.8	43.9	0.66	
		November 1		33.5	44.8	0.75	
27	Diabetes	February 23		18.2	20.7	0.88	
		February 24		21.4	31.2	0.69	After 25 grams $NaHCO_3$
		February 28		36.4	39.6	0.92	
		March 6		34.9	47.3	0.74	Complete recovery from acidemia without further alkaline therapy
Diabetics that recovered from acidemia without alkaline therapy							
28	Diabetes	January 25		40.7	36.8	0.90	
		January 27		34.1	44.5	0.77	
		January 29		39.4	48.3	0.82	Spontaneous recovery from mild acidemia
		January 30		36.2	48.3	0.75	
		February 3		42.6	43.8	0.97	
		February 9		43.9	44.5	0.99	
31	Diabetes	February 26		33.7	35.7	0.94	
		February 27		33.0	38.8	0.85	
		February 28		32.7	34.3	0.96	Spontaneous recovery from mild acidemia
		March 3		32.0	38.5	0.83	
		March 5		41.2	43.9	0.94	
		March 9		40.1	45.2	0.89	
		March 16		38.1	39.2	0.97	

TABLE 3—Continued

CASE NO.	DIAGNOSIS	DATE	TIME	ALVEOLAR CO_2	PLASMA CO_2	ALVEOLAR: PLASMA RATIO	PROCEDURE AND REMARKS
Diabetic that recovered from acidemia without alkaline therapy (Continued)							
30	Diabetes	February 9		21.1	20.3	1.04	
		February 10		23.8	30.1	0.80	
		February 11		27.1	28.4	0.95	
		February 12		27.1	31.5	0.87	Spontaneous recovery from severe acidosis
		February 13		27.1	34.8	0.78	
		February 14		23.2	32.6	0.71	
		February 16		31.6	40.3	0.78	
		February 18		32.7	40.6	0.81	
		February 20		38.4	44.8	0.86	
		February 22		34.0	41.0	0.83	
		February 26		35.9	44.1	0.81	
		February 28		36.4	41.0	0.89	
		March 3		35.6	43.1	0.83	
		March 6		35.6	45.2	0.79	
		March 9		35.9	43.9	0.82	
		March 12		29.5	41.0	0.72	
		March 15		30.2	39.2	0.77	
		March 20		34.5	42.0	0.82	
		March 24		35.8	43.1	0.83	
		April 5		30.6	45.9	0.67	

Three miscellaneous low ratios

25	Diabetes	December 30	8.30 a.m.	37.6	42.4	0.89	Fasting 14 hours
			10.30 a.m.	40.0	43.8	0.91	After small meal of carbohydrate
		January 1	8.30 a.m.	36.7	43.9	0.84	Fasting 14 hours
			12.30 p.m.	39.2	43.9	0.89	After carbohydrate feeding
			3.30 p.m.	42.4	43.4	0.98	Continued carbohydrate feeding
32	Diabetes	March 9	10.30 a.m.	40.1	40.6	0.99	Fasting
		March 15	3.00 p.m.	33.1	44.8	0.74	After carbohydrate feeding
		April 5	11.30 a.m.	41.1	43.4	0.95	Fasting 17 hours
53	Chronic gout	June 12		35.2	47.3	0.74	One day after admission during an acute attack of gout. Pain was no longer severe

The next two cases in table 3 and figures 4 and 5 illustrate the effect of the administration of adrenalin-hydrochlorid (Parke-Davis 1:1,000) intramuscularly to one normal person and one diabetic. This procedure it was discovered always produced a drop in the plasma carbonates, a fall in alveolar carbon-dioxide and usually hyperpnea.[3]

Again the alveolar curve lagged behind that of the plasma and a discrepancy was produced.

The results of exercise were just what one should have expected and are given merely as an illustration of the use of the method. There was an initial increase in CO_2 production so great that the eliminative powers of the lungs were overtaxed and the carbon dioxide tension of the alveolar air rose. When the exercise ceased the respiratory system rapidly caught up. From the beginning there was a slight increase in the H-ion concentration of the blood due to fixed acid, as is shown by the plasma curve. The immediate effect of adrenalin was very much like that of exercise except that the acidosis was entirely due to fixed acid, i.e., there was no initial rise of the alveolar CO_2. The hyperpnea and the diminution of the plasma carbonates were due to a real reduction of blood alkalinity and not to an over-ventilation in response to some abnormal respiratory stimulus. If it were a simple matter of "auspumpfung," the alveolar CO_2 should have been lower than the plasma

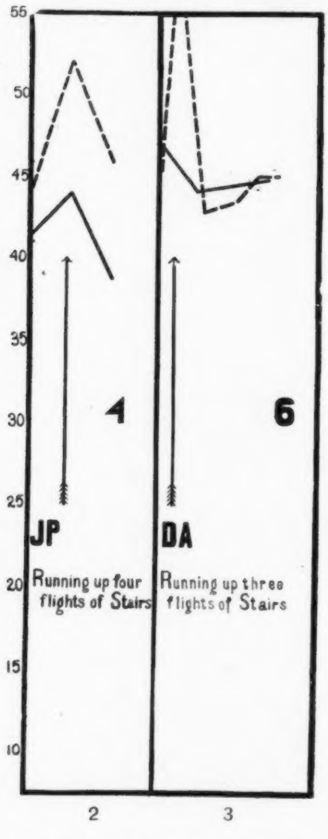

Fig. 2 and Fig. 3. The effect of exercise. Abscissa = time; ordinate = mm. Hg CO_2 tension; solid line = plasma CO_2, broken line = alveolar CO_2. The arrows indicate the time of the exercise.

[3] The detailed protocols and results of this work will be published elsewhere.

CO_2. On the other hand, if the effect of adrenalin were a carbon dioxide acidosis one should have expected an initial rise in both curves. The delay in the alveolar curve after adrenalin is probably due to the

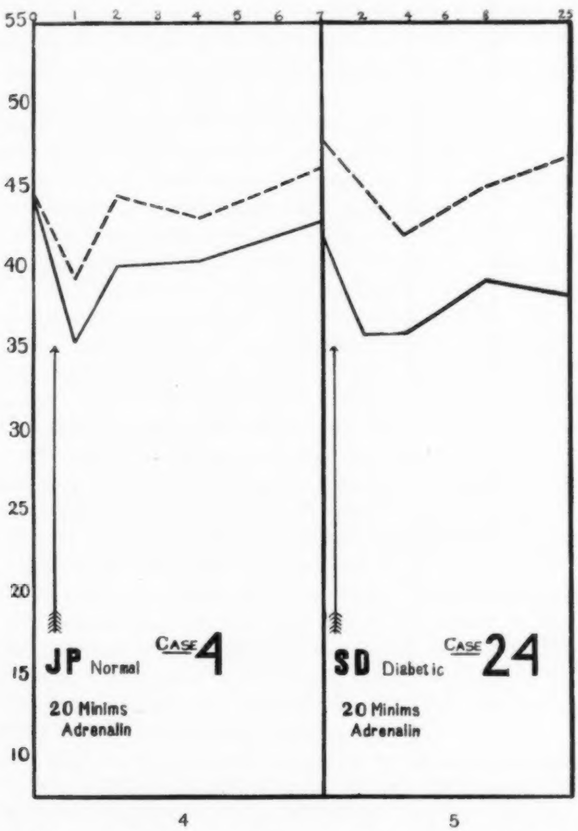

Fig. 4 and Fig. 5. The effect of adrenalin. Abscissa = time in hours; ordinate = mm. Hg CO_2 tension; solid line = plasma CO_2; broken line = alveolar CO_2. Arrows indicate time of adrenalin injection.

same cause as that after exercise, merely an undue tax on the eliminative power of the lungs.

No observations were made that showed very well the duration of this discrepancy, because it has not been possible to study any persons during the development of severe acidemia that persisted for any length of time. Some variable but suggestive data were, however, obtained. Case 5, table 3, a normal person, developed a distinctly abnormal ratio with a relatively high alveolar CO_2 after three days' starvation, when his plasma carbonates had fallen to 33.3 mm. Hg. All the other high ratios were discovered in cases of acidosis, which had presumably developed comparatively recently (table 3, cases 23, 52, 47 and 49). On the other hand, in case no. 21, a diabetic, during a drop in the plasma CO_2 from 30 to 20 mm. Hg. the alveolar CO_2 fell lower than the plasma CO_2.

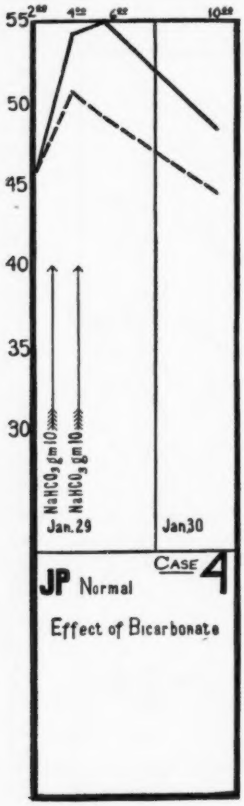

Fig. 6 The effect of bicarbonate on a normal person. Abscissa = time in hours; ordinate = mm. Hg CO_2 tension; solid line = plasma CO_2; broken line = alveolar CO_2. Arrows indicate time of bicarbonate administration.

The disagreements that occurred after recovery from acidosis or after the administration of alkali are much harder to explain but were even more striking. These comprise the last twelve cases in table 3. The first eight cases received bicarbonate of soda in varying amounts, the last four recovered from acidemia without the aid of alkaline therapy. The effect was evidently not due to any specific action of sodium bicarbonate because the results in the last cases were fully as remarkable as those in the first series. The diabetic cases were much more clear cut than the others. This might be expected because the carbonate changes were much greater and were continuous and sustained. The change of reaction in the first case, a normal person, was only moderate and was not maintained. In the nephritics there was a constant tendency for the acidemia to recur and it was impossible to tell whether at a given moment the H-ion concentration of the blood was increasing or diminishing. Obviously the last

determinations on cases 47 and 49 did not represent the results of increased alkalinity, even though they followed the administration of bicarbonate. In both cases they gave the lowest values ever obtained on these patients. Cases 48 and 50, on the other hand, were comparable to the diabetic cases. Case 50 also showed very clearly that a recurrence of acidosis was associated with a drop in the plasma carbonates out of all proportion to the alveolar CO_2. The other cases followed the same rule.

It is impossible to ascribe the disturbed ratio after an increase in blood alkalinity to any mechanism comparable to that offered above as an explanation of the change after the development of an acidemia. This would assume a rapid fixation of free CO_2 by the increased alkali of the blood. Such a reaction might occur after very rapid changes and might well explain the slight discrepancy observed in case 4, but it is inconceivable that it could persist for four weeks as in case 30, or even five days as in cases 28 and 31. The disturbance must be much more profound and is hardly susceptible of any such simple explanation.

There is nothing new in most of the work presented. The curves of exercise, the delayed equilibrium after rapid changes in blood reaction that appeared after exercise and adrenalin and after the administration of alkali have been recognized for many years (6). We have repeated these experiments by a new method merely to corroborate the work of others and by so doing to demonstrate the applicability of this simple method to a study of the state of the respiratory mechanism.

The long duration of the disturbance after recovery from acidosis has never before been studied, as far as we can discover, partly because most workers have confined themselves to one method. The few who have used more than one method have employed methods that varied so greatly in sensibility that all disagreements have been ascribed to differences in the nature of the methods used. We have chosen two methods that are in principle the same.

One of them, the alveolar CO_2, is subject to physiological influence, while the other can be affected only by changes in the actual chemical composition of the blood. The Van Slyke method determines the reaction of the plasma in so far as it is dependent upon changes in fixed acid and alkali. It is a measure of the amount of "buffer" in the plasma. The results described here would suggest that the alveolar carbon dioxide tension which has been so widely used for the same purpose was applicable only under certain conditions and was likely to fail just when it was most needed as a guide to therapy. This naturally casts considerable doubt on the value of all alveolar methods.

The failure of alveolar methods to furnish results that duplicate more accurate chemical methods has led many to discard them; others, with even less reason have discarded the Van Slyke method or other

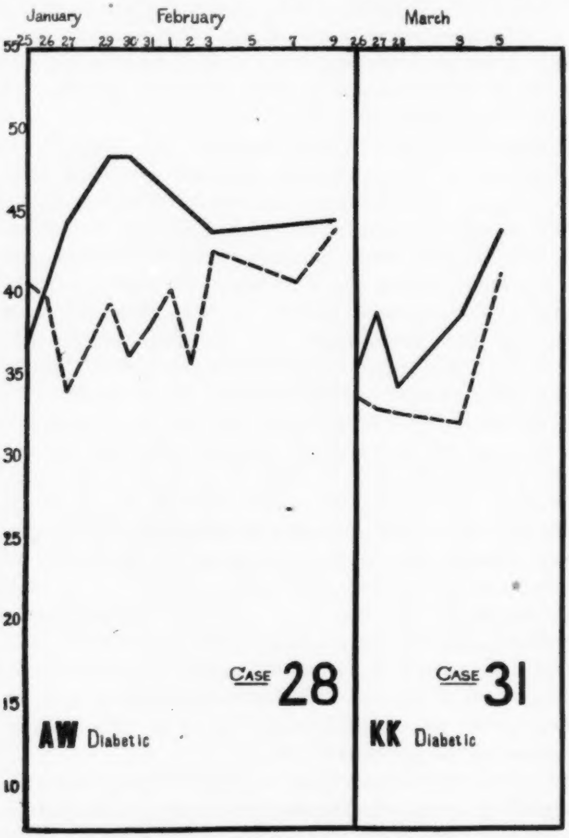

Fig. 7 and Fig. 8. Spontaneous recovery from mild acidemia in diabetes. Abscissa = time in days; ordinate = mm. Hg CO_2 tension; solid line = plasma CO_2; broken line = alveolar CO_2.

chemical methods. We have already shown that the combination may be used to distinguish between renal and cardiac dyspnea and as a measure of cardiac function. Whether the disagreements in other

cases will be of more than physiological interest depends entirely on their cause.

The only disagreements that can not be explained easily in the light of our present physiological knowledge are those of long duration occurring after alkalinization of the blood. Several possibilities present themselves. The first is, of course, errors in the determination of the alveolar carbon dioxide. This can be best discussed under two heads: errors in theory and errors in practice. Krogh and his followers have consistently held that Haldane's theory and methods were faulty. Evidence has been presented repeatedly by both sides. The controversy has involved especially the volume of the dead space. Each side has questioned the methods of the other, both for convincing reasons. There seems to be no possibility of settling the argument unless some entirely new principle is evolved. For the present purposes the discussion is more or less academic. The exact anatomical portion of the lungs from which the specimen of air was obtained is unimportant. What it was desired to study was the composition of that portion of air which is in closest contact with the blood circulating in the lungs and probably most nearly in gaseous equilibrium with this blood. The fact that the same average agreement has been found between the alveolar and plasma values at all plasma carbonate concentrations and under very different conditions of pulmonary ventilation is an indication that proper samples have been obtained.

Errors in practice would largely depend on the undue influence of personal factors. These are generally supposed to be more important when Haldane methods are used than with modifications of the Plesch method In case 21 both the Fridericia and the Plesch-Higgins method were used and gave identical results. It has been found that most patients could be easily trained to deliver specimens of alveolar air that agreed very closely. Moreover, if these discrepancies were all due to personal factors it is extraordinary that they should occur in all persons under the same conditions and not at other times.

It is possible that the cause may be the same as that to which the discrepancies found in cardiac dyspnea is ascribed: an inability to excrete CO_2. If this is so the mechanism must be very different, because no defects pointing to an anatomical pulmonary deficiency could be discovered. The vital capacity was determined in two cases and was not diminished so that the pulmonary ventilation was apparently normal. Any inability to excrete CO_2 must depend on an impermeability of the alveolar walls or circulatory changes and no evidence of

these could be found. It is easier to explain the abnormal ratios as a result of disturbances in the sensibility of the respiratory center.

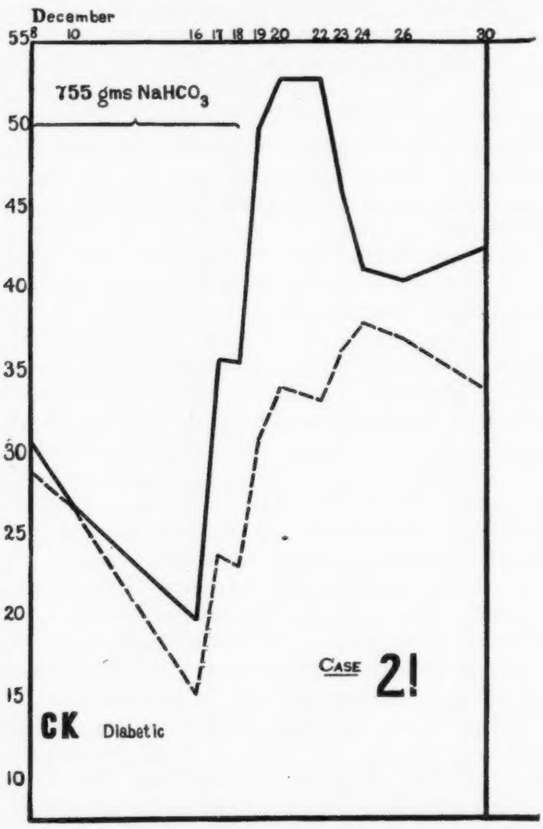

Fig. 9. Recovery from severe acidemia in diabetes under alkaline therapy. Abscissa = time in days; ordinate = mm. Hg CO_2 tension; solid line = plasma CO_2; broken line = alveolar CO_2.

Haldane and his party, on the Pike's Peak expedition, discovered that acclimatization, as expressed by the fall in alveolar carbon dioxide tension, was delayed in some cases for a few days after the arrival at high altitudes (6). On the return to sea level there was a similar delay

that was sometimes prolonged for weeks. This has been ascribed to an increased sensibility of the respiratory center to the H-ion concentration of the blood, so that the pulmonary ventilation is greater than normal at a given H-ion concentration.

There is at least one other possibility: that the respiratory center reacts to some abnormal stimulus. Winterstein (7) was the first to assert that the natural stimulus of the respiratory center was the H-ion concentration of the blood and that the effect of CO_2 on respira-

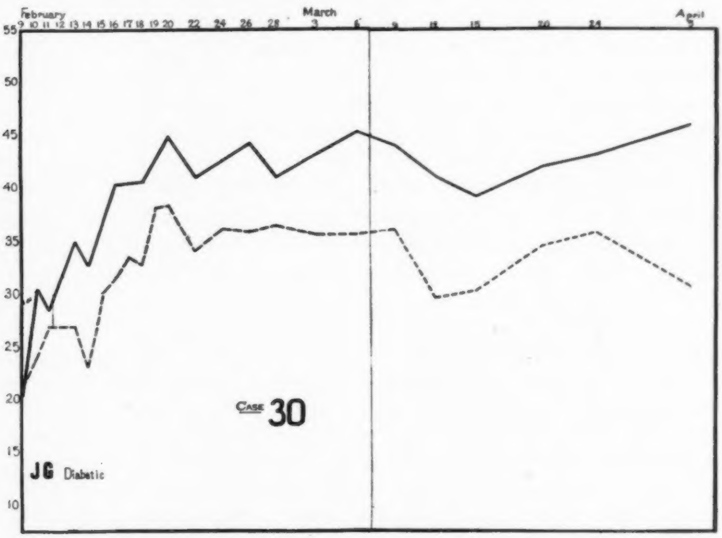

Fig. 10. Recovery from severe acidemia in diabetes without alkaline therapy. Abscissa = time in days; ordinate = mm. Hg CO_2 tension; solid line = plasma CO_2; broken line = alveolar CO_2.

tion was entirely dependent upon its ability to change the reaction of solutions. This Hasselbalch (8) corroborated with improved methods. The latter also discovered that the respiratory response was so perfectly and delicately measured that the diminution in the CO_2 tension of the blood produced by the increase in ventilation was just sufficient to compensate for the increased acidity due to fixed acid. Later work by Winterstein (9) although interpreted by him in support of his theory, seems much less clear. He discovered that after infusions of different

acids and alkalies the H-ion concentration of the blood and the changes in the pulmonary ventilation were parallel, while the alveolar CO_2 tension sometimes ran at variance to both. The difficulty in accepting this as conclusive proof is that even the most delicate electrometric methods are very much less sensitive than the respiratory center in detecting changes in blood reaction. Hooker (10) has recently studied the response of the respiratory center to acid by direct perfusion experiments. He has shown that CO_2 has a specific stimulating effect beside its effect as a weak acid. It is possible that there may be in these cases an over production of carbon dioxide.

Evidently it is most important to determine the pulmonary ventilation and the gaseous exchange. Unfortunately it was found necessary to discontinue the work for a time before this could be done. Clinical observation is peculiarly faulty as a means of determining pulmonary ventilation and such notes as we have are worth little. It is generally taught that the ventilation varies directly with the level of carbon dioxide tension in the alveolar air. If this were so the task would not be nearly so difficult, but there is an obvious absurdity in this doctrine which is borne out by some of our observations. Two other factors must have a distinct influence: (1) the rate of production of CO_2, and (2) the rate of change of the carbon dioxide tension. The exercise and adrenalin curves illustrate this perfectly. The absolute depression of the plasma carbonate concentration was very slight but the hyperpnea was extreme because the change was produced so rapidly. The observations on no. 21 were also very interesting. On admission, although the patient's plasma carbonates had fallen only to 30 mm. Hg., he showed almost typical Kussmaul breathing. This continued as long as the acidosis was increasing. During the period in which the increase in acidosis had ceased and the plasma carbonates remained between 19 and 20 mm. Hg. it was difficult to distinguish any hyperpnea clinically. It has seemed as if there was a detectable over-ventilation at the time when these patients had recovered from acidemia, but still had low alveolar carbon dioxide; but it is impossible to trust these observations or to assume a connection between the two without more accurate study.

It seems highly probable that these discrepancies may have an influence on respiratory quotients because the latter are, to a certain degree, dependent upon normal respiratory function. They should have no effect after they are established because then the carbon dioxide output must be dependent only on its rate of production, just as it is

under normal conditions. But during the production of the discrepancy there must be a disturbance of this relation. If the discrepancy is dependent on increased respiratory center sensibility there should be an apparent excess in the production of carbon dioxide and an abnormally high quotient. To prevent errors it would seem advisable to make determinations of alveolar carbon dioxide and plasma carbonates both before and after calorimetric periods in all cases in which the development or presence of an abnormal ratio might be suspected.

At the same time there must be an error in the determination of the true reaction of the blood by the Van Slyke method. Values for "buffer" and fixed acidity can be accepted, but if a respiratory center change is responsible for the disagreement of alveolar and plasma CO_2 the apparent H-ion concentration given by the Van Slyke method must be too high when the alveolar : plasma ratio is low and vice versa.

The combination of the two methods, then, offers a method for studying the degree of anatomical defects in the lungs, the sensibility of the respiratory center, the reaction of the blood as determined by fixed acid and alkaline radicals and variations due to abnormal carbon dioxide saturation.

SUMMARY

1. A comparative study of the Van Slyke method for determining plasma carbonates with the Fridericia method for alveolar CO_2 tension shows a maximum variation of 10 per cent in the alveolar : plasma ratio in all normal persons under natural conditions at rest.

2. The combination offers a simple and sensitive method for the determination of the functional state of the respiratory mechanism and the sensibility of the respiratory center to the H-ion concentration of the blood.

3. If the alveolar : plasma ratio is below 0.85 or above 1.15 some disturbance of the respiratory mechanism exists.

4. Abnormal ratios have been found to occur in cardiac dyspnea and advanced pulmonary disease with a considerable diminution of the vital capacity and are due, in these cases, to a pulmonary insufficiency that interferes with the ventilation of the blood.

5. They are also very common after rapid changes in the reaction of the blood. In these cases the alveolar CO_2 tension seems to lag behind the plasma carbonates.

6. After the rapid production of a mild acidosis this is due to the fact that the eliminative power of the lungs is overtaxed.

7. In some cases after recovery from severe acidosis the abnormal ratio may persist for weeks. This occurs whether the recovery is due to alkaline therapy or is spontaneous. The discrepancy in these cases is probably due to an increase in the sensibility of the respiratory center to the H-ion concentration of the blood.

8. This renders alveolar methods useless for the determination of blood reaction at times when such determination is most important for therapeutic purposes. It may also have an influence on respiratory quotients.

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